

ARMED FORCES RADIOBIOLOGY RESEARCH INST BETHESDA MD
ANNUAL RESEARCH REPORT, 1 JULY 1969 - 30 JUNE 1970. (U)
JUN 70
AFRRI-ARR-4

NL

AFRRI-ARR-4

$$\frac{1}{\Delta_{\text{OS}} \text{CSO}} \{r_+\}$$

2005 08 01

■

[illegible]

END
DATE
FILMED
11-81
DTIC

OTIC

AD A106080

DTIC FILE COPY

11-10-3
AD 70 4167

LEVEL III

(2)

ARR-4

**ARMED FORCES
RADIOBIOLOGY
RESEARCH INSTITUTE**

DTIC
ELECTE
OCT 26 1981
S D
E

ANNUAL RESEARCH REPORT

1 JULY 1969 — 30 JUNE 1970

Defense Atomic Support Agency, Bethesda, Maryland

Approved for public release; distribution unlimited

81 10 23

X12

All aspects of investigative programs involving the use of laboratory animals sponsored by DoD components are conducted according to the principles enunciated in the "Guide for Laboratory Animal Facilities and Care", prepared by the National Academy of Sciences - National Research Council.

14

AFRR1 - ARR-4

6

**ANNUAL RESEARCH REPORT,
1 JULY 1969 - 30 JUNE 1970.**

1130 Jun 70

12
671

**ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
DEFENSE ATOMIC SUPPORT AGENCY
BETHESDA, MARYLAND 20014**

Approved for public release; distribution unlimited

6341

FOREWORD

This report summarizes the scientific accomplishments of the Armed Forces Radiobiology Research Institute (AFRRI) for the period 1 July 1969 to 30 June 1970. During this period acceptance testing of the Institute's electron linear accelerator was very satisfactorily completed and the machine became available for use. Of the three types of radiation (electrons, x rays and neutrons) that can be produced by this machine, it was decided that the electron had the greatest potential for AFRRI's biological research program. Much of the available time on the machine was used for electron dosimetry and beam expansion studies.

Construction was completed on 36,000 square feet of additional space for the Institute. This space included areas for a conference room, a library, a cobalt-60 source, and numerous small laboratories.

The Institute hosted two meetings during this period. On 18 and 19 November 1969 the DASA (Semiannual) Medical Coordination Conference was held in the new conference room. Attendees included members from other DASA-supported laboratories and from universities performing research on Radiation-Induced Incapacitation and Performance Decrement.

The 88th Meeting of the Joint Medical Research Conference was held at AFRRI on 8 May 1970. Papers were presented by the staff on selected research results.

Accession For	
MHS GRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
Distribution/	
Availability Codes	
List	Avail and/or Special
A	



HUGH B. MITCHELL

Colonel, USAF, MC
Director

TABLE OF CONTENTS

	Page
THE RELATIVE EFFECTIVENESS OF FISSION NEUTRONS FOR INCAPACITATION. George, R. E., Verrelli, D. M., Chaput, R. L. and Thorp, J. W.	1
INVESTIGATION OF INCAPACITATING DOSES OF RADIATION IN THE LARGER MAMMALS. Chaput, R. L. and Kovacic, R. T.	3
THE EFFECT OF PARTIAL BODY SHIELDING ON THE INCAPACITATION AND LETHALITY RESPONSE OF LARGER MAMMALS. Thorp, J. W. and Young, R. W.	6
THE PATHOPHYSIOLOGICAL RESPONSE OF LARGER MAMMALS TO MULTIPLE EXPOSURES OF MIXED GAMMA-NEUTRON RADIATION. West, J. E.	10
THE RELATIVE EFFECTIVENESS OF FISSION NEUTRONS FOR GASTROINTESTINAL DEATH IN MINIATURE SWINE, Jones, S. R.	14
DRUG RESPONSIVENESS IN THE POSTIRRADIATION ANIMAL. Strike, T. A., Turns, J. E., Doyle, T. F. and Miletich, D. J.	17
THE BEHAVIORAL PERFORMANCE OF THE UNRESTRAINED MONKEY FOLLOWING MIXED GAMMA-NEUTRON IRRADIATION. Curran, C. R.	21
BEHAVIORAL INCAPACITATION STUDIES OF THE RESTRAINED MONKEY (<u>MACACA</u> <u>MULATTA</u>). McFarland, W. L. and Young, R. W.	25
IDENTIFICATION OF PROMINENT SITES OF RADIATION INJURY AND THEIR RELATIONSHIP TO BEHAVIOR. Turbyfill, C. L. and Roudon, R. M.	29
HIPPOCAMPAL ELECTRICAL ACTIVITY AND VOLUNTARY MOTOR MOVEMENT IN THE RAT. McFarland, W. L.	32
RECOVERY AND RESIDUAL INJURY OF THE HEMATOPOIETIC SYSTEM IN IRRADIATED MAMMALS. Baum, S. J. and Wyant, D. E.	34
MOLECULAR STUDIES OF CELLULAR AND SUBCELLULAR DAMAGE IN THE IRRADIATED ANIMAL. Catravas, G. N.	36
EFFECT OF IONIZING RADIATION ON LIPID PEROXIDATION IN MAMMALIAN CELL MEMBRANES, Skidmore, W. D. and Catravas, G. N.	39
EFFECTS OF IONIZING RADIATIONS ON BIOSYNTHESIS OF COMPLEX PROTEINS. Evans, A. S.	41
EFFECTS OF IONIZING RADIATION ON THE ULTRASTRUCTURE OF MAMMALIAN TISSUES, René, A. A.	46
EFFECTS OF IONIZING RADIATION ON IMMUNE RESPONSES. Eikman, E. A. and Bowser, R. T.	48
ALTERATION OF THE CIRCULATORY AUTOREGULATION OF THE SMALL INTES- TINE DURING THE DEVELOPMENT OF THE GASTROINTESTINAL RADIATION SYNDROME. Kabal, J.	51
HEPATIC CYSTICERCOSIS IN A MOUSE COLONY. Balk, M. W. and Jones, S. R.	53
NEUTRON FLUENCE TO KERMA FACTORS AND MASS ENERGY TRANSFER COEFFICIENTS FOR MATERIALS IN REACTOR NEUTRON FIELDS. Leonard, B. E.	56
INDEX TO PRINCIPAL INVESTIGATORS	59

THE RELATIVE EFFECTIVENESS OF FISSION NEUTRONS FOR INCAPACITATION

Principal Investigators: *R. E. George, D. M. Verrelli, R. L. Chaput and J. W. Thorp*

Technical Assistance: *E. L. Barron*

The objective of this research is to determine the relative effectiveness of fission neutrons for performance decrement and incapacitation in several larger mammalian species.

Miniature pigs, trained by shock avoidance conditioning to traverse a shuttle-box on cue, were whole-body irradiated in a nuclear reactor-produced neutron field (incident neutron to gamma ratio of 10). Their postirradiation performance was compared to that of pigs similarly irradiated in either a gamma ray field from the same reactor (incident neutron to gamma ratio of 0.06) or a mixed neutron-gamma field (incident neutron to gamma ratio of 0.4). The dose rate at the midline of the brain was approximately 2000 rads/min for all irradiations. The midbrain doses ranged from 1500 to 36,500 rads.

The response of the pigs to supralethal doses from the neutron field notably differed from their response to similar doses from the gamma ray field.¹ Early performance decrement, early transient incapacitation, and immediate permanent incapacitation occurred following much lower doses from the gamma ray field than from the neutron field. Postirradiation performance was better for the neutron field than for the gamma ray field (Figure 1). At doses greater than 11,000 rads, survival times of the neutron-irradiated pigs were on the average much longer than those of the gamma-irradiated pigs (Figure 2). Thus, the gamma rays were markedly more effective than the fission neutrons for producing the severe central nervous system damage manifested by pronounced early performance decrement and death within 48 hours. The relative effectiveness of the neutron field was 0.23 using the reactor-produced gamma ray field as the reference radiation and the 50 percentile midbrain dose (ED_{50}) for early performance decrement as the biological end point.

Data from the mixed gamma-neutron irradiations support the findings from the neutron and the gamma irradiations in that the response to a given dose was consistently intermediate between the responses obtained with equivalent doses from the neutron field and from the gamma ray field (Figures 1 and 2).

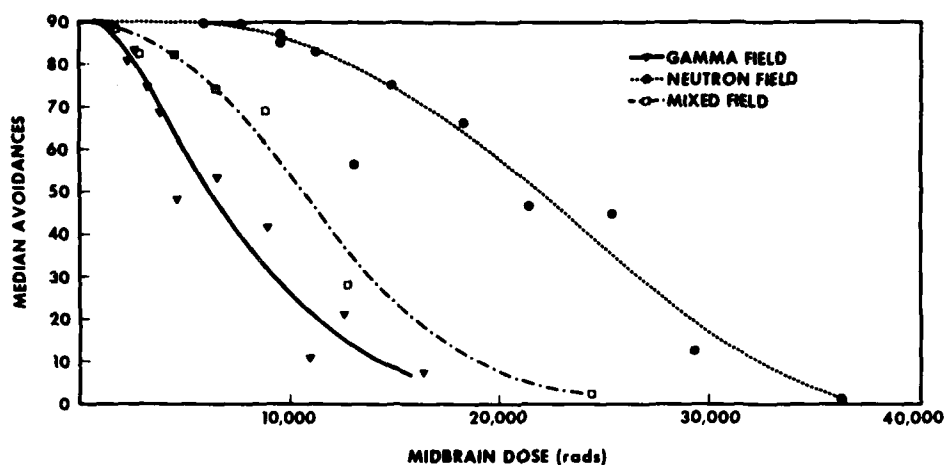


Figure 1. Median number of shock avoidances during first 30 minutes postirradiation.

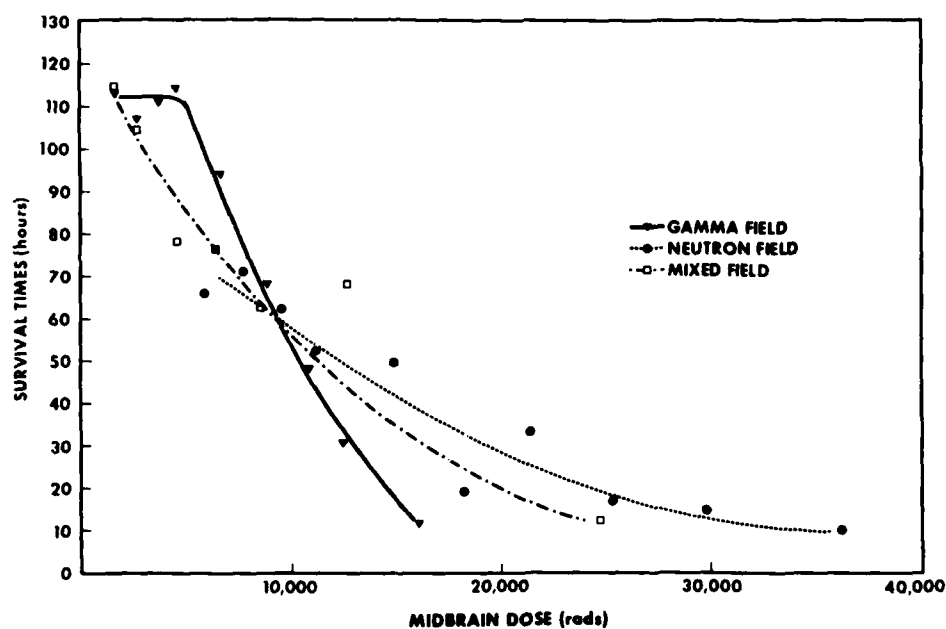


Figure 2. Mean survival times.

REFERENCE

1. George, R. E., Chaput, R. L., Verrelli, D. M. and Barron, E. L. The relative effectiveness of fission neutrons for miniature pig performance decrement. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR71-2, 1971 (in press).

INVESTIGATION OF INCAPACITATING DOSES OF RADIATION IN THE LARGER MAMMALS

Principal Investigators: *R. L. Chaput and R. T. Kovacic*

Technical Assistance: *E. L. Barron, W. W. Wolfe, J. K. Warrenteltz, M. E. Flynn,
N. L. Fleming and T. K. Dalton*

In the evaluation of incapacitation and performance decrement response of several mammalian species to supralethal doses of pulsed mixed gamma-neutron radiation, it was shown that the response of the miniature pig is reduced when such doses are fractionated.¹ The time-dose relationship was then investigated.² The objectives were to determine how the responses of miniature pigs after a dose given in two fractions depended upon the interval between fractions and upon the size of the first fraction.

The pigs were trained to traverse, on cue, a two-chambered shuttlebox. Six groups of five to eight pigs received a 4400-rad midline tissue dose of pulsed mixed gamma-neutron radiation followed 1/2, 1, 5, 15, 24, or 51 hours later by a second dose of equivalent magnitude. Two other groups of eight pigs received a lower initial dose (1700 or 3400 rads) followed 1 hour later by a second dose of 4800 to 5000 rads. In addition, unfractionated doses of either 4500 or 8600 rads were given to two groups of six pigs.

Regardless of the time interval between the two 4400-rad doses, performance was generally better after the second dose than after the first (Table I). Early transient incapacitation (ETI), accompanied always by convulsions, and occasionally by coma, consistently occurred after the first dose but was infrequent after the second. When early transient incapacitation did occur after the second dose, it was generally shorter than after the first dose and accompanied by ataxia and disorientation; convulsions and coma were very infrequent. Because of this reduced response to the second 4400-rad dose, performance after an 8800-rad fractionated dose was markedly better than after an 8600-rad unfractionated dose. However, as the first dose was reduced to 3400 or 1700 rads, early transient incapacitation accompanied by occasional convulsions was observed after the subsequent 4800- to 5000-rad dose. Furthermore, early transient incapacitation after the second dose was longer and was more frequently accompanied by convulsions when the initial dose was only 1700 rads.

Mean survival times of all groups of pigs receiving fractionated doses were similar to the mean survival time of pigs receiving a single 4500-rad dose and significantly longer than the mean survival time of pigs receiving a single 8600-rad dose (Table II).

Table I. Miniature Pig Performance after Fractionated and Unfractionated Doses of Radiation: Effects of Changes in Time Interval between Two 4400-Rad Dose Fractions

Pig #	Time between fractions (h)	Fractionated dose (4400 + 4400 rads)					
		Duration of ETI (min)		Onset of acceptable* performance (min postirradiation)		Number of avoidances during first 30 min (90 possible)	
		Fraction 1	Fraction 2	Fraction 1	Fraction 2	Fraction 1	Fraction 2
1	1/2	+	3.5‡	+	10	+	56
2		+	0	+	7.5	+	81
3		+	0	+	0	+	85
4		+	0	+	2.5	+	80
5		+	0	+	25	+	57
6		+	0	+	2.5	+	85
7	1	9.7	0	15	0	44	90
8		16.3	0	20	7.5	29	82
9		1.3	0	10	0	73	85
10		2.5	0	10	0	68	87
11		1.5	0	7.5	0	78	90
12		1.2	0	10	0	65	84
13		‡	‡	‡	‡	0	0
14	5	1	1	10	5	74	76
15		0	0	10	10	73	79
16		22	5	25	15	19	63
17		1	0	10	5	78	85
18		0	0	5	0	84	86
19		0	0	7.5	5	73	83
20	15	0	0	0	5	89	83
21		0	0	0	10	88	81
22		17	0	20	2.5	28	83
23		15	30‡	25	**	32	7
24		1.4	0	5	5	80	85
25		12.1	0	25	5	35	80
26		15	0	20	10	36	75
27		‡	‡	‡	‡	0	0
28	24	6	0	10	5	54	85
29		9	0	15	0	49	90
30		2	0	7.5	0	72	87
31		30.5	0	45	5	8	78
32		12	0	20	5	38	86
33	51	11	3.9‡	20	7.5	38	66
34		8.8	5‡	20	5	56	68
35		31.5	10.8	90	30	3	37
36		1.3	2.1‡	10	2.5	68	76
37		15.2	0.5‡	25	5	30	81
Unfractionated dose (8600 rads)							
38		9		20		38	
39		30		120		4	
40		40		**		0	
41		240		**		0	
42		10		15		43	
43		60		**		0	

* Proficiency of 90 percent avoidance

† Pigs were not tested between fractions 1 and 2

‡ No convulsions were observed during ETI

§ Permanently incapacitated immediately postirradiation

** Between ETI and permanent incapacitation, pig never achieved acceptable performance

Table II. Miniature Pig Survival Time after Fractionated and Unfractionated Doses of Radiation*

Fractionated Dose								
Size of dose fractions (rads)	4400 + 4400						1700 + 4800	3400 + 5000
Hours between fractions	1/2	1	5	15	24	51	1	1
Survival time (h)	Number of pigs (mean survival time)							
<2	1(1.25)	3*(0.5)			1*(0.1)			
8-39				2(22)	1(38)			
40-118	5(85)	6(69)	6(90)	6(56)	4(75)	5(77)	8(70)	8(54)
Unfractionated dose								
Size of dose (rads)	4500		8600					
Survival time (h)	Number of pigs (mean survival time)							
<2	1(0.1)		1(1.5)					
8-39			4(25)					
40-118	5(71)		1(71)					

* Calculated from the initial dose

* Two pigs in the 1-hour and one pig in the 24-hour groups died before the second 4400-rad dose was delivered

REFERENCES

1. Chaput, R. L. and Kovacic, R. T. Miniature pig performance after fractionated doses of ionizing radiation. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR69-22, 1969.
2. Chaput, R. L. and Kovacic, R. T. Miniature pig performance after fractionated doses of radiation: Time-dose relationships. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR70-5, 1970.

THE EFFECT OF PARTIAL BODY SHIELDING ON THE INCAPACITATION AND LETHALITY RESPONSE OF LARGER MAMMALS

Principal Investigators: J. W. Thorp and R. W. Young

Technical Assistance: E. L. Barron, T. K. Dalton, N. L. Fleming, M. E. Flynn,
J. K. Warrenfeltz and W. W. Wolfe

The early incapacitation observed in dogs and miniature pigs that received high supralethal whole-body doses of pulsed mixed gamma-neutron radiation was prevented by shielding the head. Head irradiation (trunk shielded) caused the same type of incapacitation as was observed in animals that received comparable whole-body doses.

The main goals of this research project have been to: (1) determine whether the relationship between head irradiation and the occurrence of early incapacitation in monkeys is similar to the relationship observed for dogs and pigs; and (2) to characterize the dose-response relationship for partial body irradiated monkeys,² dogs and pigs.¹

Forty-one male and thirty female monkeys (*Macaca mulatta*) were trained by shock avoidance conditioning to work a simultaneous visual discrimination problem. Trained subjects received either 2500, 4500 or 10,000 rads (midline tissue doses) of pulsed mixed gamma-neutron radiation. Within each dose group some animals were head shielded, some were trunk shielded, and some were not shielded. The midline tissue dose behind the shield (at the middle of the head or chest) was less than 8 percent of the midline tissue dose to the same point without the shield in place. If a subject made 90 or more correct responses during a 100-trial test period, its performance was considered acceptable; fewer than 90 correct responses were considered a performance decrement. Results are presented in Table III and Figures 3-5. All unshielded monkeys and many shielded monkeys suffered early incapacitation after irradiation. Within dose groups at 2500 rads and at 10,000 rads, the incapacitation was about equally severe for unshielded, head-shielded and trunk-shielded subjects; however, it generally was more severe at 10,000 rads than in similarly irradiated monkeys that received 2500 rads. At 4500 rads, early incapacitation did not occur in all shielded subjects, and, if it did occur in a shielded monkey, recovery occurred sooner than in most unshielded subjects at 4500 rads. There was some indication that, generally, males could perform better than females after irradiation.

Following this dose-response relationship observed in monkeys, research was conducted to better characterize the dose-response relationship for partial body irradiated dogs and pigs.

Beagles and miniature pigs were exposed individually to a pulse of mixed gamma-neutron radiation. Either the head or trunk was shielded, and the midline tissue dose behind the shield (at the middle of the head or trunk) was less than 7 percent of the

dose to the same point when no shield was in place. After exposure, the presence or absence of clinical signs of central nervous system damage and the ability to maintain an upright position in a slowly rotating box were used to evaluate the dogs' response. Miniature pigs were evaluated by measuring their performance of a learned, shock avoidance task in a two-chambered shuttlebox. Early incapacitation did not occur in head-shielded dogs or pigs that received up to 25,000 rads or 13,000 rads, respectively, to the middle of the trunk (Tables IV and V).

Table III. Monkey Performance during First 2 Hours after Irradiation

Midline tissue dose (rads)				2500			4500			10,000		
Shielding				None	Head	Trunk	None	Head	Trunk	None	Head	Trunk
Subject				Total correct responses (600 possible)								
Male												
1				227	60*	1	123*	494	408	5*	4*	77
2				269	369	313	226	535	409	6*	91*	193
3				399	464	350	410	546	532	7*	535	229
4				512	562	488	413	569	570	440		
5				531	574*	577	426	582*	593*	513		
MEAN				388	406	346	320	545	502	194	210	166
Female												
1				7*	6	142	4*	478	290			
2				19	71	224	10*	532	323			
3				266	118	374	17*	578*	471			
4				421	429	443	130*	582*	505			
5				454	574	503	360	584*	574			
MEAN				233	240	337	104	551	433			
COMBINED MEAN				311	323	342	212	548	468			

* Died during 2-hour test period

+ Had no performance decrement during 2-hour test period

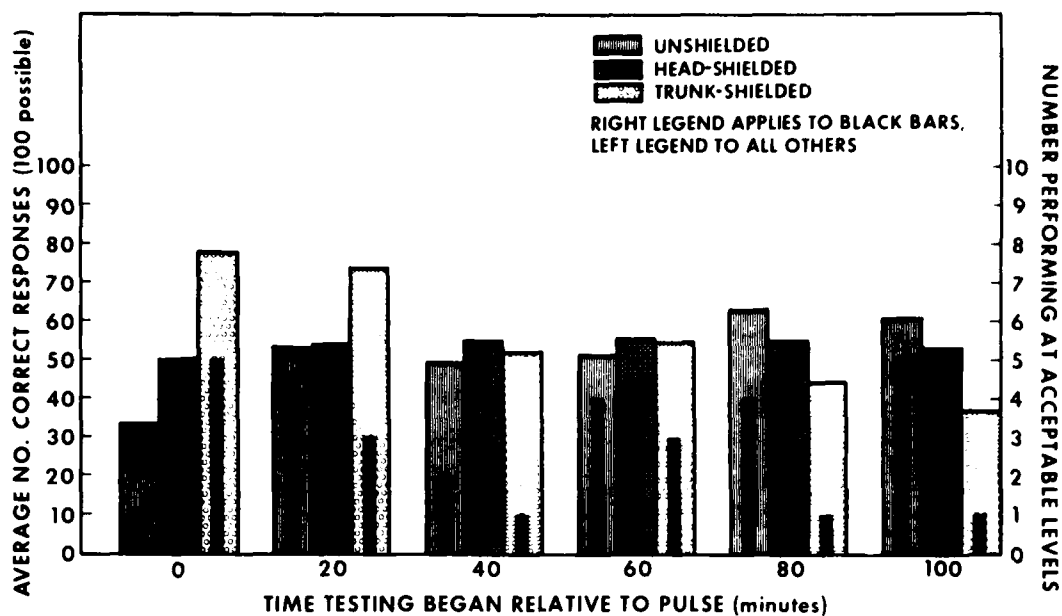


Figure 3. Monkey performance after irradiation (male and female; 2500 rads).

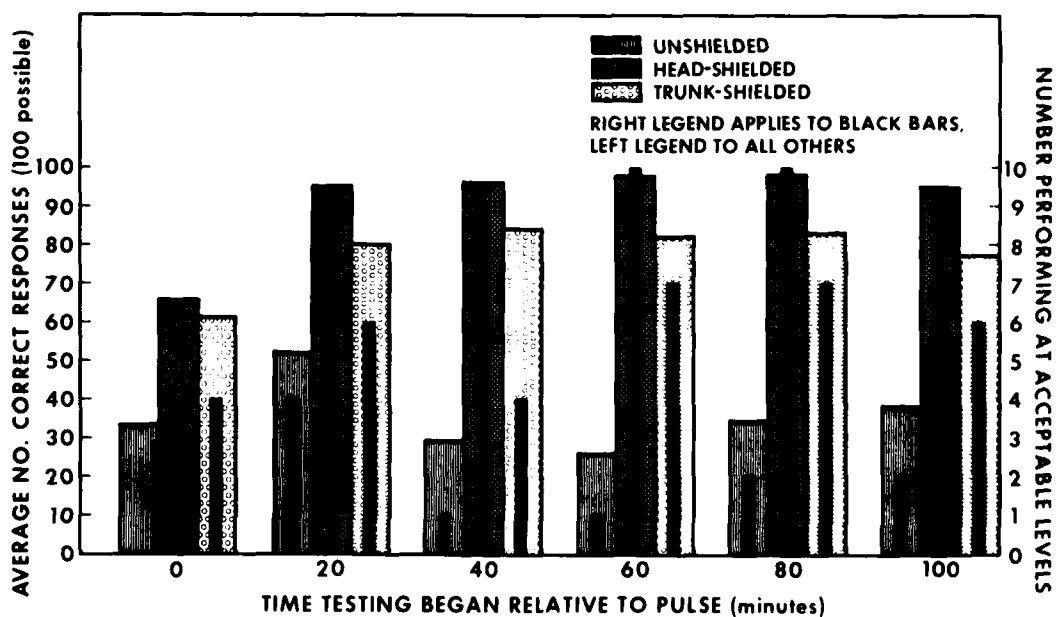


Figure 4. Monkey performance after irradiation (male and female; 4500 rads).

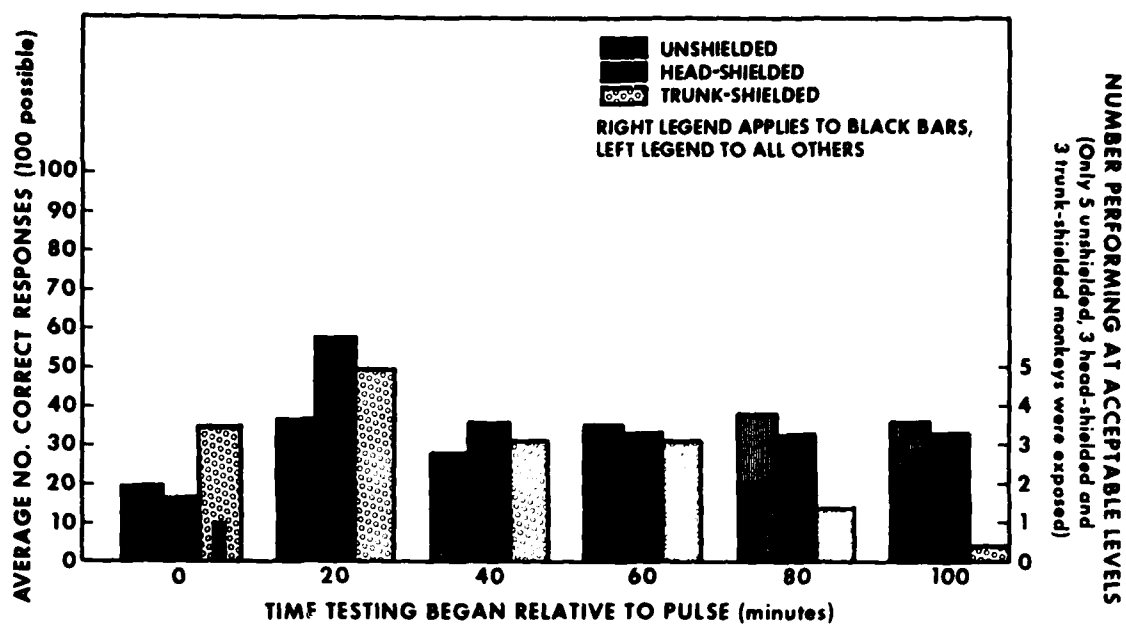


Figure 5. Monkey performance after irradiation (male; 10,000 rads).

Table IV. Beagle Response to Partial Body Irradiation
(8 per group)

Midline tissue dose (rads)		Early incapacitation	Survival time (hours)	
Head	Trunk		\bar{x}	$s \bar{x}$
shielded	5,000	no	89	8
shielded	10,000	no	55	10
shielded*	19,000	no	45	5
shielded*	25,000	no	26	1
5,000	shielded	no	294	6
10,000	shielded	yes	168	23
14,000*	shielded	yes	26	6
22,000*	shielded	yes	16	2

* Previously reported in Aerospace Medicine 40:759, 1969

Table V. Miniature Pig Response to Partial Body Irradiation

Midline tissue dose (rads)		Number of subjects	
Head	Trunk	Total	Early incapacitation
3,000*	shielded	4	1
6,000*	shielded	4	4
13,000*	shielded	8	7
shielded	6,000	8	0
shielded*	13,000	8	1

* Previously reported in Aerospace Medicine 41:379, 1970

REFERENCES

1. Thorp, J. W. Beagle and miniature pig response to partial body irradiation: Dose relationships. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Technical Note TN70-5, 1970.
2. Thorp, J. W. and Young, R. W. Monkey performance after partial body irradiation: Dose relationships. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR70-11, 1970.

◆◆◆◆◆◆◆◆◆◆

THE PATHOPHYSIOLOGICAL RESPONSE OF LARGER MAMMALS TO MULTIPLE EXPOSURES OF MIXED GAMMA-NEUTRON RADIATION

Principal Investigator: *J. E. West*

Collaborator: *S. R. Jones*

Technical Assistance: *F. A. Mitchell, J. P. Vagher, D. F. Trainor and G. D. Lee*

The objective of this research is to quantitate and compare responses of bone marrow cell renewal systems and 30-day lethality in several mammalian species

following single and multiple exposures to mixed gamma-neutron radiation at doses in the LD_{50/30} range (hematopoietic syndrome).

Male beagles were bilaterally exposed to mixed gamma-neutron radiation. The dose rate was 20 rads/min for all irradiations and the midline tissue doses were approximately 215, 225 and 240 rads. At each dose approximately one-half of the animals received a single irradiation while the others were given one-fourth the dose each day for 4 consecutive days (24 hours apart). Serial rib marrow aspirates and peripheral blood samples were studied for 32 days after irradiation.¹

Tables VI-VIII show a comparison of mean group postirradiation response data after single and fractionated exposures in each of the three dose groups. In the 215-rad dose group (Table VI) there was a greater degree of hematopoietic injury reflected in most end points, including lethality, for the beagles receiving the fractionated exposure.

Table VI. Comparison of Bone Marrow, Hematology, and Other Parameters following the same Midline Tissue Dose (215 versus 213 rads) given as Single versus Fractionated Bilateral Exposures to Mixed Gamma-Neutron Radiations in Adult Male Beagles

End point	Parameters*					
	Nucleated marrow cells (per mm ³ x 10 ⁻³)	WBC's (per mm ³ x 10 ⁻³)	Platelets (per mm ³ x 10 ⁻³)	Coagulation time* (min)	Reticulocytes (percent)	Body temperature (degrees F)
Preirradiation value	119/125	6.5/7.2	266/284	12.6/7.9	0.5/0.4	101.7/102.1
Percent preirradiation value at day 1	53/36	44/49	69/53	103/178	56/18	100.2/99.2
Time to maximum depression (days)	21/21	14/18	18/18	18/14 [‡]	5/8	18/18
Percent preirradiation value at maximum depression	8/6	15/10	1/2	143/240 [‡]	0/0	101.7/101.7
Time of beginning recovery (days)	25/25 (4)	25/21 (4)	21/21 (4)	21/25 (4)	25/25 (4)	21/21 (4)
Percent of preirradiation value at termination (32 days)	75/70 (4)	81/74 (4)	32/43 (4)	109/174 (4)	459/512 (4)	100/99.8 (4)
Lethality response						
Single exposure 0/6						
Fractionated exposure 2/6 Mean survival time 21.5 days						

* Single exposure data given first in each column -- expressed as group mean values for six dogs per group unless otherwise noted in parentheses

* Coagulation time by Lee-White method. Difference in preirradiation values due to difference in tube size used, i.e., 13 mm x 100 mm versus 10 mm x 75 mm

‡ Coagulation time at maximum (days)

§ Percent preirradiation value at maximum change

Table VII. Comparison of Bone Marrow, Hematology, and Other Parameters following the same Midline Tissue Dose (223 versus 226 rads) given as Single versus Fractionated Bilateral Exposures to Mixed Gamma-Neutron Radiations in Adult Male Beagles

End point	Parameters*					
	Nucleated marrow cells (per mm ³ x 10 ⁻³)	WBC's (per mm ³ x 10 ⁻³)	Platelets (per mm ³ x 10 ⁻³)	Coagulation time† (min)	Reticulocytes (percent)	Body temperature (degrees F)
Preirradiation value	125/119	7.3/9.9	238/286	8/13	0.5/0.7	101.8/101.7
Percent preirradiation value at day 1	36/44	98/43	101/95	102/104	46/1	100.1/99.6
Time to maximum depression (days)	14/21	14/21	14/21	(3) 21/18†	8/1	(2) 18/18
Percent preirradiation value at maximum depression	7/5	10/11	2.5/0.4	(3) 233/134‡	0/4	(2) 102.0/101.5
Time of beginning recovery (days)	(3) 18/25	(3) 18/25	(3) 18/25	(2) 28/21	(2) 25/12	(2) 25/25
Percent of preirradiation value at termination (32 days)	(1) 79/61	(1) 52/53	(1) 80/65	(1) 158/106	(1) 220/290	(1) 101.0/100.7
Lethality response						
Single exposure 2/4 Mean survival time 18.5 days						
Fractionated exposure 0/6						

* Single exposure data given first in each column -- expressed as group mean values; single exposure data on four dogs except where noted otherwise in parentheses; fractionated exposure, six dogs

† Coagulation time by Lee-White method. Difference in preirradiation values due to difference in tube size used, i.e., 10 mm x 75 mm versus 13 mm x 100 mm

‡ Coagulation time at maximum (days)

§ Percent preirradiation value at maximum change

Table VIII. Comparison of Bone Marrow, Hematology, and Other Parameters following the same Midline Tissue Dose (241 versus 235 rads) given as Single versus Fractionated Bilateral Exposures to Mixed Gamma-Neutron Radiations in Adult Male Beagles

End point	Parameters*					
	Nucleated marrow cells (per mm ³ x 10 ⁻³)	WBC's (per mm ³ x 10 ⁻³)	Platelets (per mm ³ x 10 ⁻³)	Coagulation time† (min)	Reticulocytes (percent)	Body temperature (degrees F)
Preirradiation value	170/163	9.8/8.2	282/263	12.7/12.6	0.9/0.5	102.0/101.8
Percent preirradiation value at day 1	35/25	36/38	87/105	101/115	37/14	99.5/100
Time to maximum depression (days)	8/14	(5) 14/18	(5) 14/18	(3) 18/140	5/6	(5) 14/18
Percent preirradiation value at maximum depression	6/6	(5) 5/10	(5) 0/1	(3) 156/134‡	0/0	(5) 102.0/101.3
Time of beginning recovery (days)	(1) 21/18	(3) 18/21	(1) 21/21	(1) 21/25	(1) 25/25	(1) 21/25
Percent of preirradiation value at termination (32 days)	(1) 36/44	(1) 36/59	(1) 67/54	(1) 103/104	(1) 130/216	(1) 99.7/99.5
Lethality response						
Single exposure 5/6 Mean survival time 15.8 days						
Fractionated exposure 0/6						

* Single exposure data given first in each column -- expressed as group mean values; single exposure data on six dogs except where noted otherwise in parentheses; fractionated exposure, six dogs

† Coagulation time by Lee-White method; used 13 mm x 100 mm tubes

‡ Coagulation time at maximum (days)

§ Percent preirradiation value at maximum change

In the 225- and 240-rad dose groups (Tables VII and VIII) the reverse was found between the single and fractionated groups. The greater response after the single exposure was reflected by more severe changes in most hematopoietic and clinical indices measured, as well as in the lethality response. Radiation-induced changes in total nucleated marrow cell counts after single and fractionated exposures at the highest dose are shown in Figure 6.

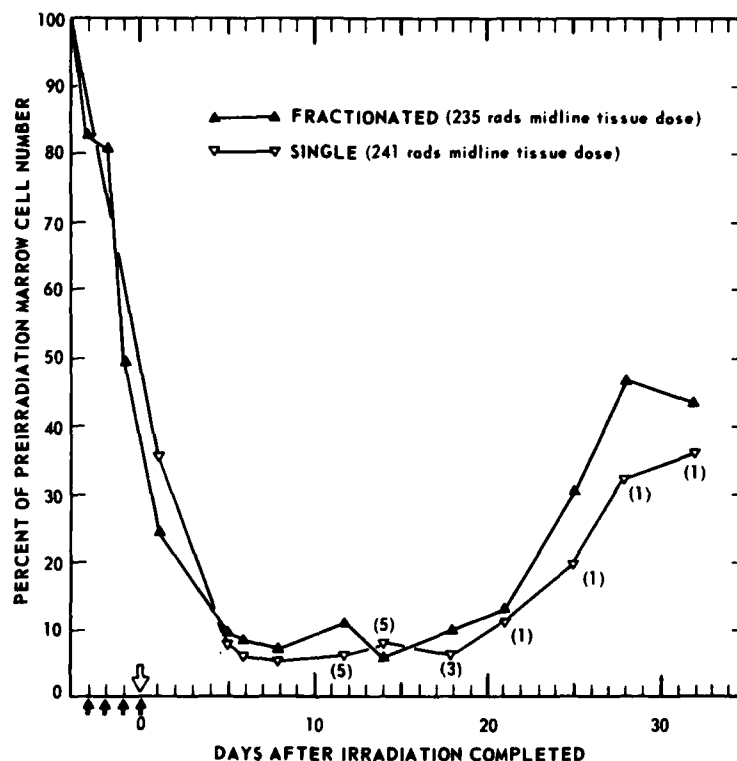


Figure 6. Response of total nucleated marrow cells per $\text{mm}^3 \times 10^{-3}$ expressed as percent of pre-irradiation numbers after single and fractionated mixed gamma-neutron exposures in adult beagles. The numbers in parentheses indicate the number of animals when less than six.

Complete necropsies were performed on decedents and survivors. Histopathologic examination of bone marrow was emphasized. The in-depth quantitative study of the progressive changes occurring within cell renewal compartments in bone marrow and peripheral blood and selected clinical parameters revealed marked damage to the target hematopoietic system. Lethality was attributed to common sequelae occurring secondary to compromised bone marrow granulocytopoiesis and thrombocytopoiesis.

Data analysis revealed quantitatively similar changes during the degenerative, nadir and regenerative response phases within the immature marrow and mature peripheral blood cell compartments of the granulocytic and erythrocytic cell renewal systems after single and fractionated exposures at each dose.

REFERENCE

1. West, J. E., Mitchell, F. A. and Vagher, J. P. Serial rib marrow aspiration technique and myelogram for adult beagles. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Technical Note TN71-1, 1971 (in press).

THE RELATIVE EFFECTIVENESS OF FISSION NEUTRONS FOR GASTROINTESTINAL DEATH IN MINIATURE SWINE

Principal Investigator: S. R. Jones

Collaborators: R. E. George, D. M. Verrelli and J. E. West

Technical Assistance: G. D. Lee

The relative effectiveness of fission neutrons for intestinal damage is being investigated in miniature pigs.

Miniature pigs were bilaterally irradiated either in a neutron field (incident neutron to gamma ratio of 5) or a gamma field (incident gamma to neutron ratio of 15) from the AFRRI-TRIGA reactor. For both fields the dose rate at the midline of the pigs was 250 rads/min and uniform (class A) irradiations to the gastrointestinal tract were achieved. Midline tissue doses from the neutron field ranged from 360 to 1970 rads; survival times were from 3.6 to 11 days. Midline tissue doses from the gamma field ranged from 605 to 2630 rads and survival times from 4.5 to 11 days.

Median lethal doses for gastrointestinal death ($LD_{50/7.5}$) were determined by correlation of survival times with gross and microscopic lesions and subsequent probit analysis. The median lethal dose of fission neutrons was 425 rads and of gamma rays was 870 rads (Tables IX and X). Relative effectiveness of the neutrons, expressed as the quotient of the gamma LD_{50} /neutron LD_{50} , was 2.0. In addition, least squares fitted regression lines were calculated for survival times versus dose for both the neutron- and the gamma-irradiated pigs (Figure 7). Midline tissue doses (MTD) corresponding to 7.5 days survival on these fitted curves also were used to calculate the relative effectiveness of the neutrons. The MTD at 7.5 days survival was 430 rads for neutron- and 920 rads for gamma-irradiated pigs. The relative effectiveness of the neutrons, expressed as the quotient of the gamma MTD/neutron MTD, was 2.1. The four highest neutron doses (Table IX) and the two highest gamma doses (Table X) were deleted from the least squares fitted regression lines because they were in the nonlinear portion of the response versus log dose curve.

Pig #	Midline tissue dose (rads)	Survival time (days)	Bacteria* in blood or lungs	Weight preexposure (kg)	Weight lost (kg)	Necropsy lesions primarily GI* or Hemo*
1	360	10.8	G+ cocci	31.7	1.2	Hemo
2	370	11.0	S culture	30.7	1.5	Hemo
3	385	10.7	S culture	32.9	1.4	Hemo
4	410	10.1	E, S	30.8	1.6	Hemo
5	410	6.9 R†	S culture	33.5	3.1	GI
6	410	8.6	G- rods	31.6	2.1	Hemo - GI
7	415	8.7	G+ cocci	31.7	2.7	Hemo - GI
LD ₅₀ /7.5 425						
8	430	8.6	E, P culture	30.1	1.8	Hemo - GI
9	430	5.2 R	none in culture	30.0	5.3	GI
10	435	9.1	P culture	30.1	1.3	Hemo - GI
11	440	6.7 R	P, S	30.0	2.0	GI
12	440	6.7 R	A culture	29.8	3.0	GI
13	460	3.9 R	P culture	32.9	5.7	GI
14	470	5.4 R	none in culture	20.4	3.0	GI
15	480	4.8 R	unknown	21.3	1.5	GI
16	480	5.1 R	G+ cocci	25.0	2.2	GI
17	505	4.8 R	none in culture	33.2	6.5	GI
18	520	5.5 R	none in culture	36.5	6.5	GI
19	520	3.6 R	Proteus	37.6	3.6	GI
20	530	4.8 R	unknown	27.1	1.5	GI
21	530	4.5 R	none in culture	30.8	4.0	GI
22	985	5.5 R	none in tissue	28.3	7.0	GI
23	985	4.9 R	none in tissue	28.7	5.0	GI
24	1965	5.6 R	G- rods	29.0	6.3	GI
25	1970	4.8 R	P culture	30.3	6.0	GI

* A = Aerobacter; E = E. coli; P = Pseudomonas; S = Streptococci;
 G+ cocci = gram-positive coccoid bacteria in histologic sections of lung;
 G- rods = gram-negative bacilli in histologic sections of lung
 † GI = gastrointestinal tract; Hemo = hemopoietic system; gi = gastrointestinal tract with only minimal to moderate lesions
 ‡ R = responded, died within 7.5 days

Table IX. Neutron-irradiated Pigs

Table X. Gamma-irradiated Pigs

Pig #	Midline tissue dose (rads)	Survival time (days)	Bacteria* in blood or lungs	Weight preexposure (kg)	Weight lost (kg)	Necropsy lesions primarily GI* or Hemo*
1	605	8.6	unknown	39.0	2.4	Hemo - GI
2	610	11.1	G+ cocci	41.0	4.5	Hemo
3	650	11.1	G+ cocci	32.2	3.7	Hemo
4	650	8.5	G+ cocci	31.7	1.8	Hemo - GI
5	760	7.4 R†	none in tissue	31.0	3.7	GI - Hemo
6	760	7.9	E, S	30.2	2.2	Hemo
7	770	7.9	S culture	32.7	4.4	Hemo - GI
8	815	8.5	G+ cocci	32.2	5.5	Hemo
LD ₅₀ /7.5 870						
9	890	6.9 R	P, S	32.0	3.6	GI - Hemo
10	910	6.7 R	unknown	41.0	5.0	GI - Hemo
11	920	9.0	unknown	41.0	4.0	Hemo - GI
12	920	7.8	P, S	32.0	3.6	Hemo - GI
13	1020	4.4 R	none in tissue	31.7	3.3	GI
14	1045	5.9 R	P culture	37.2	4.5	GI - Hemo
15	1040	7.3 R	G+ cocci	35.0	3.7	GI
16	1210	5.5 R	G+ cocci	42.0	6.0	GI
17	1220	6.9 R	unknown	44.5	6.2	GI
18	1290	7.4 R	E, S	44.3	8.3	GI
19	1300	6.7 R	S	40.4	6.7	GI
20	2615	6.1 R	G+ cocci	38.7	8.8	GI
21	2630	6.3 R	G+ cocci	39.7	8.6	GI

* E = E. coli; P = Pseudomonas; S = Streptococci; G+ cocci = gram-positive coccoid bacteria in histologic sections of lung
 † GI = gastrointestinal tract; Hemo = hemopoietic system; gi = gastrointestinal tract with only minimal to moderate lesions
 ‡ R = responded, died within 7.5 days

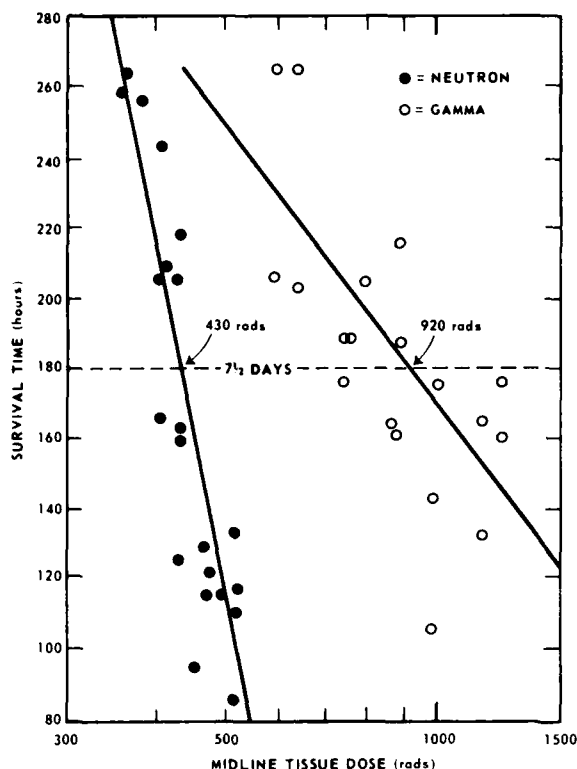


Figure 7. Survival times of neutron- and gamma-irradiated miniature pigs (least squares fitted regression lines).

Based on gross lesions and preliminary histopathologic correlation, the relative segmental radiosensitivity of the gastrointestinal tract of the miniature pigs in this study from most to least sensitive is as follows:

1. ileum
2. cecum, proximal (spiral) colon and jejunum
3. stomach
4. duodenum and distal colon

Sixteen of twenty-three neutron-irradiated pigs and 15 of 17 gamma-irradiated pigs had postirradiation bacterial proliferation (Tables IX and X). These bacteria were either cultured from samples of cardiac blood or lung tissue or were detected with Gram's stain in histologic sections of lungs. Bacteriologic studies were not conducted on two of the neutron- and four of the gamma-irradiated pigs and are listed in Tables IX and X as unknown.

DRUG RESPONSIVENESS IN THE POSTIRRADIATION ANIMAL

Principal Investigators: T. A. Strike, J. E. Turns, T. F. Doyle and D. J. Miletich

Technical Assistance: S. L. Bradley, R. H. Crutcher and W. G. Ewald

The effect of sympathomimetic amines on radiation-induced tolerance to pentobarbital in the rat³ and the effect of vasopressor drugs and an antihistamine on post-irradiation hypotension and incapacitation in the monkey^{2,4} were investigated.

Low doses of radiation are known to increase the normal background electrical activity of the nervous system of animals. This intensified activity generally results in an increased release of a neurotransmitter. Mice receiving sublethal doses of radiation have been shown to have an enhanced pentobarbital tolerance.¹ This increased tolerance appears related to the increased release of neurotransmitter. The ability of irradiated rats to tolerate pentobarbital was measured after treatment with amphetamine and/or reserpine. Male Sprague-Dawley rats were given 250-, 500-, or 1000-rad midline tissue doses of 300 kVp x rays. Twenty-four hours postirradiation the acute toxicity of sodium pentobarbital was determined in these animals. Rats receiving 500 and 1000 rads demonstrated an increased tolerance to sodium pentobarbital, but no change in toxicity was observed in animals given 250 rads (Table XI).

Table XI. The Effects of X Ray, Amphetamine and Reserpine Administration on Pentobarbital Acute Toxicity in the Rat

Regimen	Number of rats	Toxic dose of pentobarbital mg kg ⁻¹ \pm S. E.
Control	29	87.2 \pm 5.5
Control, fasted 48 h	12	85.7 \pm 5.2
Irradiated (24 h after 250 rads)	15	85.6 \pm 4.8
Irradiated (24 h after 500 rads)	12	*107.6 \pm 5.4
Irradiated (24 h after 1000 rads)	12	*106.3 \pm 3.5
Amphetamine treated	21	*105.7 \pm 4.8
Amphetamine treated (24 h after 1000 rads)	18	76.5 \pm 5.0
Reserpine treated	7	85.9 \pm 7.1
Reserpine and Amphetamine treated	9	91.2 \pm 4.8
Reserpine treated (24 h after 1000 rads)	10	91.1 \pm 5.9
Reserpine and Amphetamine treated (24 h after 1000 rads)	11	84.7 \pm 2.9

* p < 0.05 (treatment group compared with control group)

This radiation-induced increase in pentobarbital tolerance was similar in degree to that observed in unirradiated rats treated with amphetamine, a known barbiturate antagonist. However, when rats received amphetamine as well as 1000 rads of x rays no increased tolerance to sodium pentobarbital was observed. Four days of reserpine treatment prior to irradiation also abolished the radiation-induced increase in sodium pentobarbital tolerance. These results suggest that increased central norepinephrine levels are responsible for the radiation-induced increase in tolerance to pentobarbital.

The effect of vasopressor drugs on the early hypotension and incapacitation observed in monkeys following a 4000-rad pulsed dose of mixed gamma-neutron radiation was investigated. Seven monkeys received only the 4000-rad dose of radiation and served as controls. Norepinephrine or angiotensin was administered to five other monkeys by intravenous infusion from 5 minutes prior to irradiation until death, except that infusion was temporarily interrupted several times after irradiation of the animals. Blood pressure was continuously monitored in all animals. Drug-induced blood pressure elevation prior to irradiation did not prevent the characteristic early postirradiation fall in mean blood pressure, but severity of the resulting hypotension was reduced (Figure 8). Cessation of drug infusion after irradiation resulted in a rapid drop in blood pressure with the animal becoming incapacitated when mean blood pressure fell below a critical level of about 50 mm Hg. After drug infusion was resumed, consciousness returned as the blood pressure rose above the critical level. This sequence was repeated several times before the animal failed to respond to drug administration and died abruptly in a hypotensive state. Five other monkeys were given a single intravenous injection of ephedrine or amphetamine 1 hour before irradiation; these animals received no further treatment. Ephedrine administration appeared to be as effective at maintaining satisfactory postirradiation blood pressure as norepinephrine and angiotensin.

An antihistamine, chlorpheniramine maleate, was used in monkeys to ameliorate the severe hypotension and to prevent signs of the early transient incapacitation (ETI) frequently associated with supralethal doses of ionizing radiation. Twenty-five monkeys (*Macaca mulatta*) were given a 4000-rad pulsed dose of mixed gamma-neutron radiation. Eight of the animals served as controls and received only normal saline injections; 10 animals were each injected with 10 mg of the antihistamine 30 minutes before irradiation; and a third group of seven animals each received 10 mg of antihistamine 60 minutes before irradiation plus 10 mg of antihistamine 30 minutes before irradiation. Blood pressure was monitored from time of injection until death, and clinical symptoms were recorded until 1 hour after irradiation. All but one of the antihistamine-treated animals remained alert and responsive to auditory stimuli with no evidence of ETI. The control animals became unconscious and unresponsive. The mean arterial pressure (MAP) of the antihistamine-treated and the control animals began to fall within 1 minute and at 2 minutes reached minimum values for the antihistamine-treated animals (Figure 9). The MAP of the control animals continued to drop, reaching a minimum at 5 minutes. The MAP of the control animals never recovered to the preirradiation value; however, the MAP of the antihistamine-treated

monkeys recovered to greater than preirradiation levels. The average survival time of the antihistamine-treated animals was significantly greater than that of the control animals.

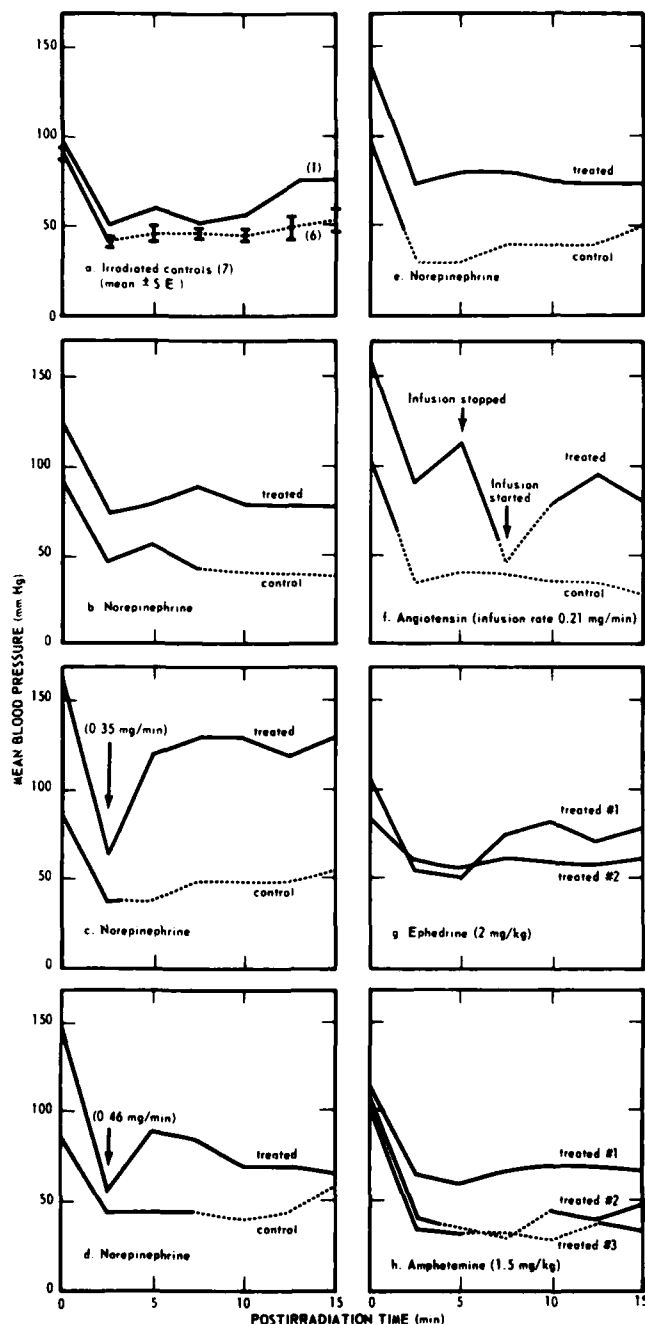


Figure 8. Mean blood pressure of monkeys after a 4000-rad pulsed dose of mixed gamma-neutron radiation. Norepinephrine infusion rate was 0.23 mg/min except where shown (c and d). Dashed lines indicate incapacitation.

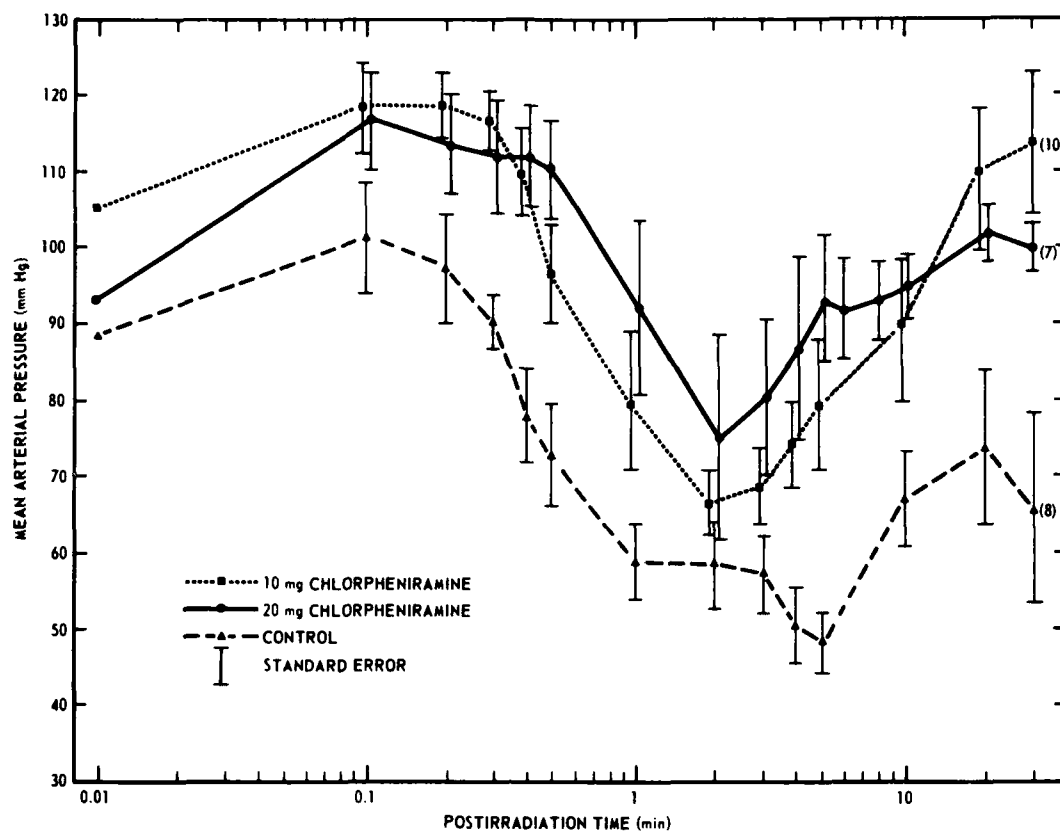


Figure 9. Postirradiation blood pressure changes in monkeys treated with an antihistamine then given 4000 rads of mixed gamma-neutron radiation.

REFERENCES

1. Barnes, C. D. Central nervous system drugs and X-irradiation: their interactive effects. *Radiation Res.* 30:351-358, 1967.
2. Doyle, T. F., Turns, J. E. and Strike, T. A. Effect of an antihistamine on early transient incapacitation of monkeys subjected to 4000 rads of mixed gamma-neutron radiation. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR70-9, 1970.
3. Miletich, D. J., Bradley, S. L. and Strike, T. A. The effect of sympathomimetic amines on radiation-induced tolerance to pentobarbital in the rat. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR69-27, 1969.

4. Miletich, D. J. and Strike, T. A. Alteration of postirradiation hypotension and incapacitation in the monkey by administration of vasopressor drugs. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR70-1, 1970.

THE BEHAVIORAL PERFORMANCE OF THE UNRESTRAINED MONKEY FOLLOWING MIXED GAMMA-NEUTRON IRRADIATION

Principal Investigator: C. R. Curran

Technical Assistance: D. W. Conrad and R. W. Young

To evaluate the effects of a 2000-rad midline tissue dose of pulsed gamma-neutron radiation on the behavior of unrestrained monkeys (*Macaca mulatta*), 14 animals were trained to operate levers and pressplates mounted on the four walls of an open chamber. After irradiation, each animal's performance was monitored for 8 hours.

On the basis of performance during the first 2 hours postirradiation, unrestrained monkeys' individual performance curves showed that five animals performed the assigned task at or near base-line levels of efficiency, five animals experienced periods of performance decrement, transient incapacitation or nonperformance and then recovered to near base-line levels, and four animals went into extended periods of incapacitation early in the period and generally did not recover before death (Figures 10-12).

For the unfettered monkey of this study, the incidence of the initial performance decrement was similar to that observed with restrained monkeys. The mean performance (percentage of correct responses) of the 14 unfettered subjects is presented in Figure 13. For purposes of comparison, the same information is also presented for 13 chaired primates exposed to a 2500-rad midline tissue dose of gamma-neutron radiation. Both groups show a performance decrement in the first 10 - 20 minutes postirradiation followed by partial recovery. However, recovery is more complete for the chaired animals and the differences between the two groups remain relatively stable for the remaining 4-1/2 hours. The mean group performance differences between the restrained and unrestrained animals are statistically significant ($p < 0.05$).

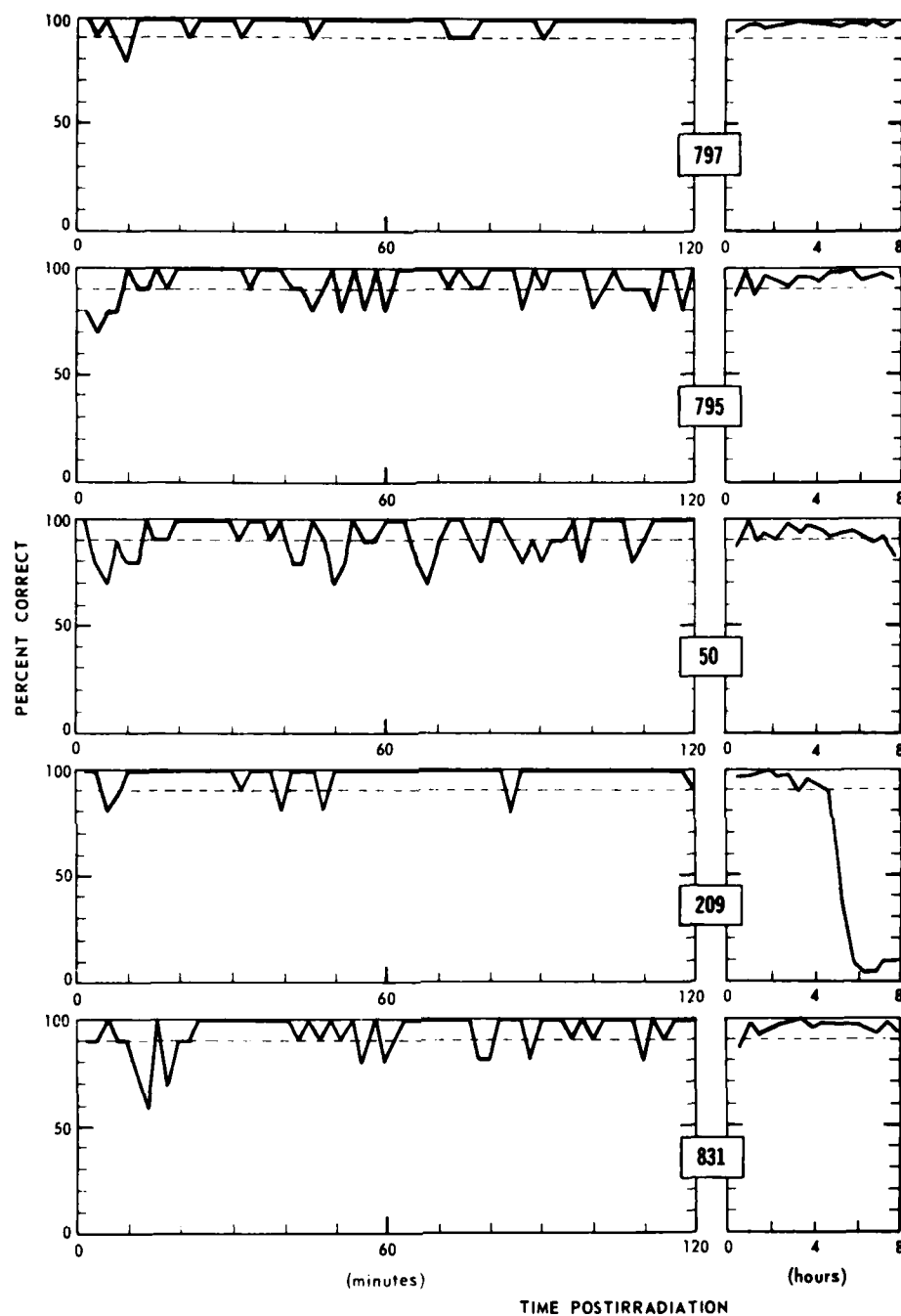


Figure 10. Percent correct responses postirradiation for unrestrained monkeys (2000 rads). The left graph presents 10-trial means for the first 2 hours. The right graph shows mean performance for 100-trial sessions across the 8-hour testing period. Criterion performance is indicated by the dashed line.

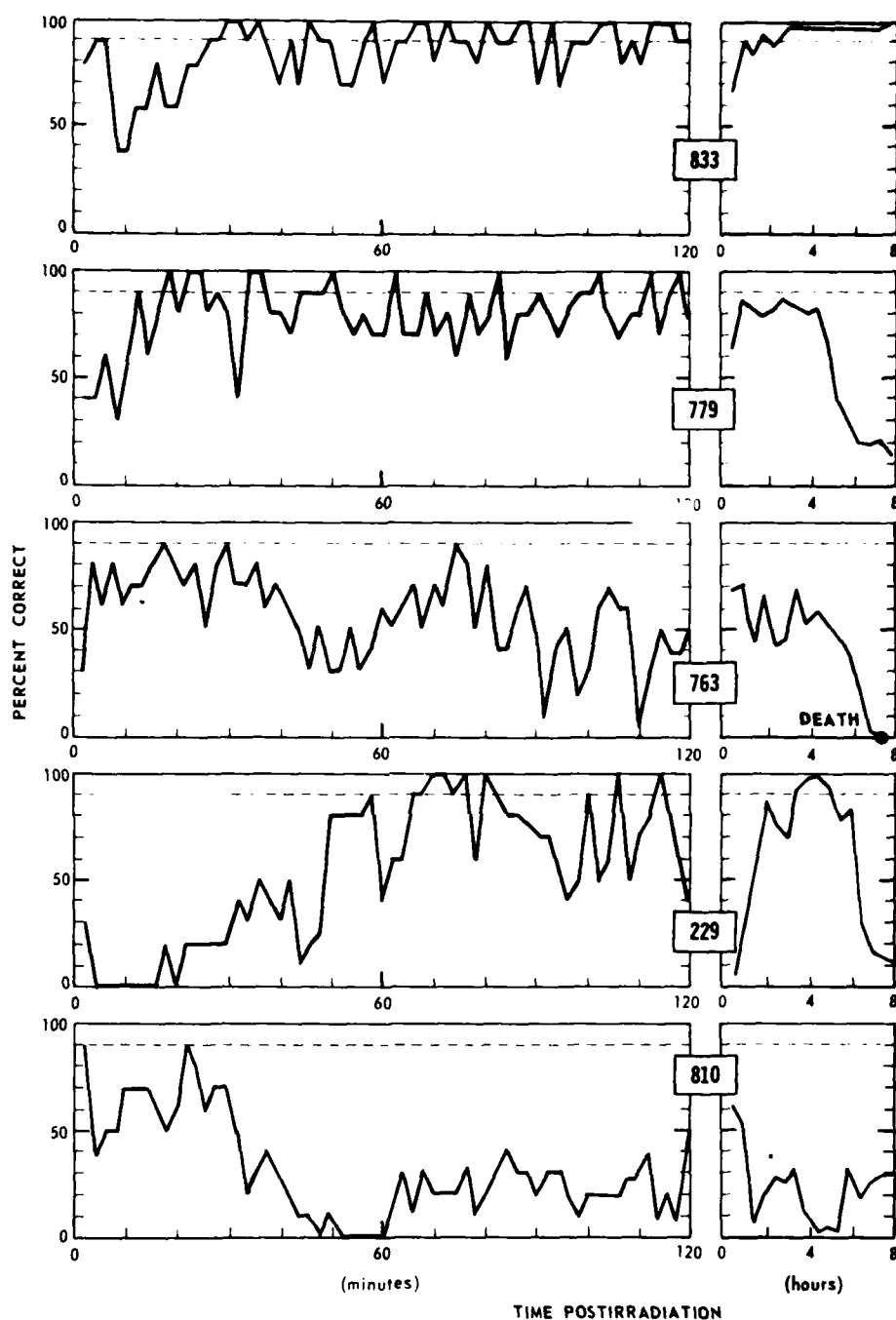


Figure 11. Percent correct responses postirradiation for unrestrained monkeys (2000 rads). The left graph presents 10-trial means for the first 2 hours. The right graph shows mean performance for 100-trial sessions across the 8-hour testing period. Criterion performance is indicated by the dashed line.

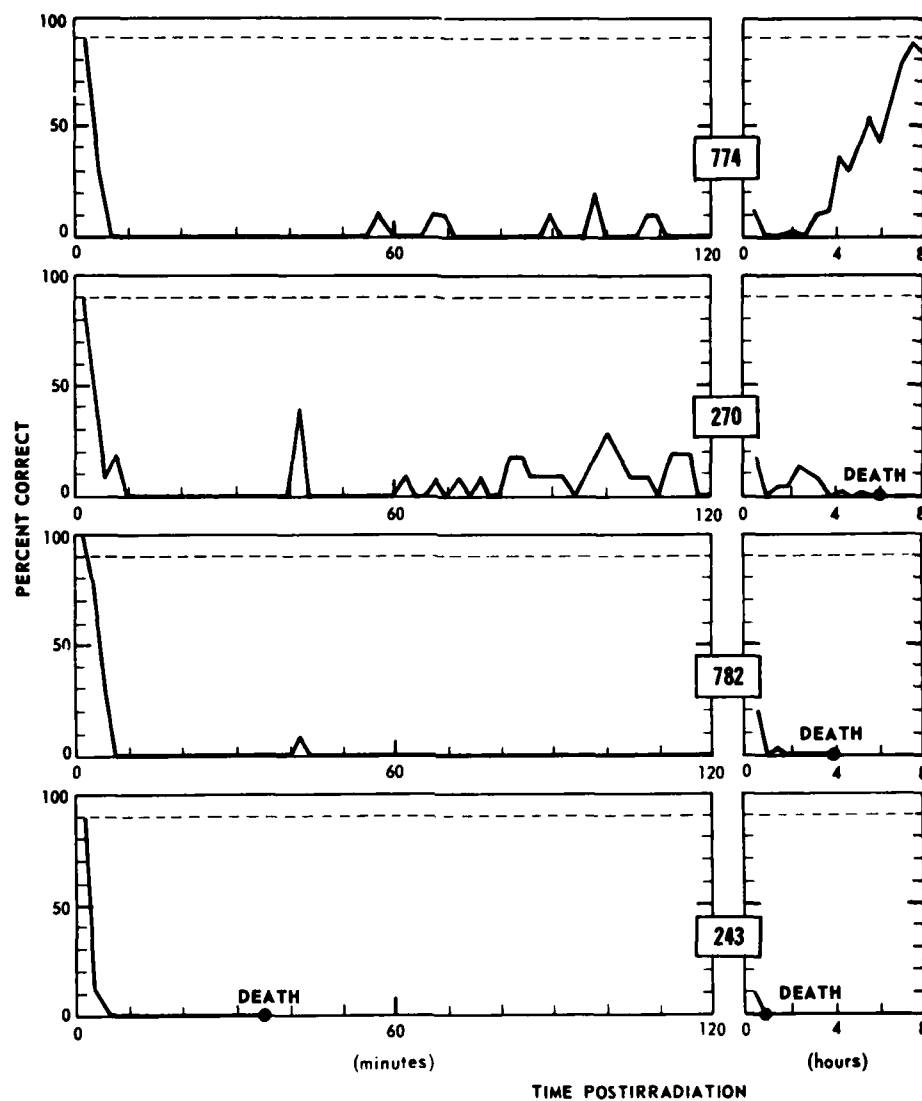


Figure 12. Percent correct responses postirradiation for unrestrained monkeys (2000 rads). The left graph presents 10-trial means for the first 2 hours. The right graph shows mean performance for 100-trial sessions across the 8-hour testing period. Criterion performance is indicated by the dashed line.

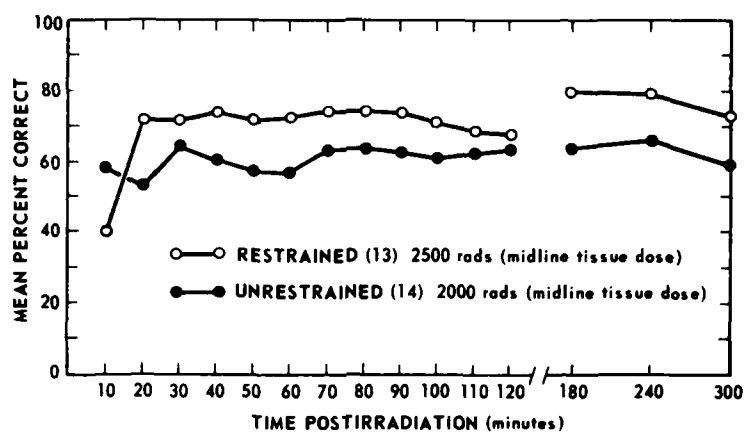


Figure 13. Group mean performance of restrained and unrestrained primates.

◆◆◆◆◆◆◆◆◆◆

BEHAVIORAL INCAPACITATION STUDIES OF THE RESTRAINED MONKEY (*MACACA MULATTA*)

Principal Investigators: *W. L. McFarland and R. W. Young*

Technical Assistance: *J. F. Lee and Q. H. Shelton*

The objective of this research was to study the behavioral incapacitation of restrained monkeys after single and fractionated doses of mixed gamma-neutron radiation.

The effect of 15,000 rads of pulsed gamma-neutron radiation on the performance capabilities of restrained monkeys was investigated² since information previously reported was largely concerned with doses of 10,000 rads or less. Seven male monkeys were used in this study. The behavioral task was a shock motivated visual discrimination task. The performance data for the seven animals are presented in Figure 14. Five of the monkeys were unable to perform within 5 minutes postirradiation and remained in a state of total nonperformance until death. The two remaining animals experienced periods of severe performance decrement followed by a return of performance capability which approached the preirradiation value. This performance capability deteriorated rapidly at 58 and 88 minutes postirradiation in these two animals, after which time the subjects failed to perform until death. The median survival time for all animals was 1 hour and 18 minutes.

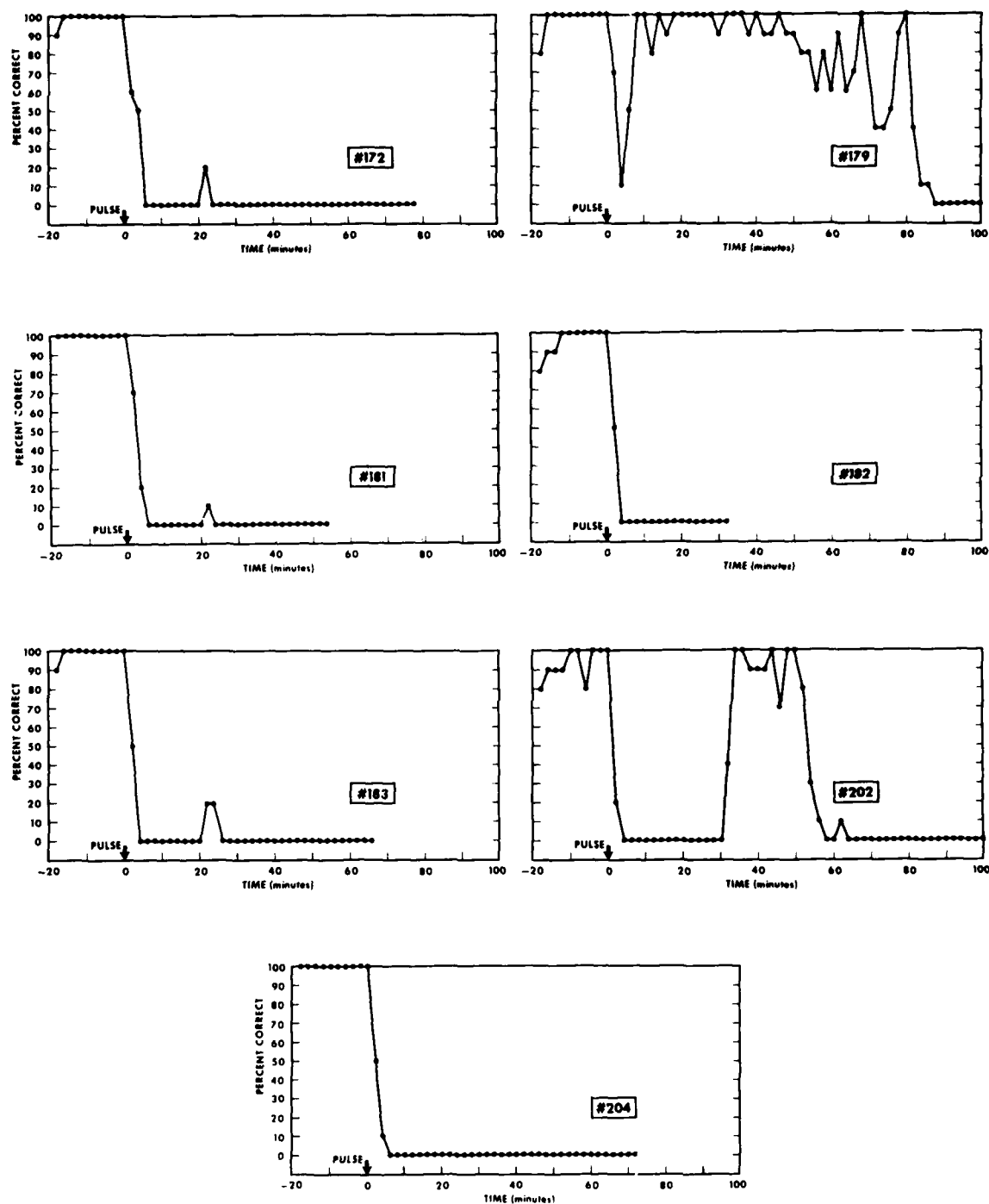


Figure 14. Percent correct performance postirradiation. During each 20-minute interval, 100 trials and a 3-minute rest period were presented. Each point on the graph is the average of 10 trials.

The effect of 1500 rads of pulsed mixed gamma-neutron radiation was also investigated. Six monkeys were used in this study. The postirradiation performance curve for this group is presented in Figure 15. There was an immediate performance decrement (performance below 90 percent correct) after exposure. The mean duration of the decrement was 11 minutes. All of the subjects had returned to preirradiation performance levels by 25 minutes after exposure. The median recovery time was 10 minutes. After recovery, performance remained at or near preirradiation levels until shortly before death.

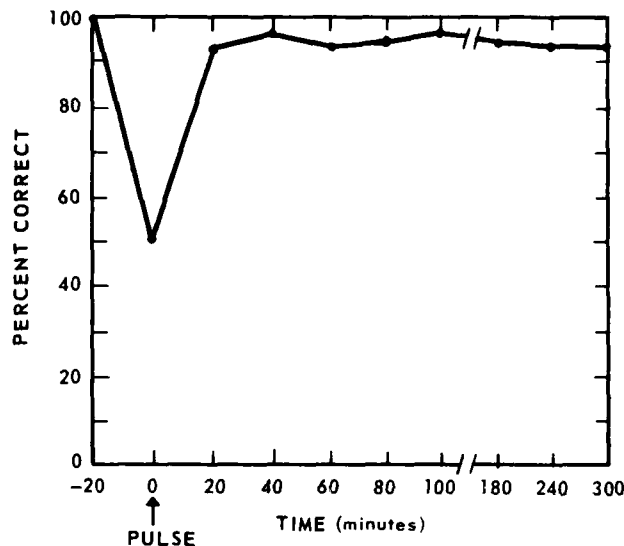


Figure 15. Mean postirradiation performance for six monkeys receiving a single whole-body pulsed dose of 1500 rads (midline tissue dose).

In the study of behavioral incapacitation of restrained monkeys after fractionated doses of mixed gamma-neutron radiation two areas were investigated: (1) two pulses of equal magnitude, and (2) two pulses of unequal magnitude.

A study of 10 monkeys exposed to two pulses of 2500 rads of mixed gamma-neutron radiation separated by 40 minutes was completed. The group data presented for these subjects in Figure 16 indicate that the decrement in performance after the second pulse is more severe than that following the first pulse.

A study employing 10 monkeys receiving 1700 rads of pulsed mixed gamma-neutron radiation followed 6 hours later by a 3500-rad pulse was completed. Figure 17 illustrates the data obtained from these animals. The greater performance decrement after pulse two than after pulse one is similar to the results obtained when two 2500-rad pulses were separated by 40 minutes (Figure 16). The results of these studies differ from the results previously reported¹ for two 2500-rad pulses separated by 6 hours, where the effect of the second pulse was less detrimental to performance than was the first pulse.

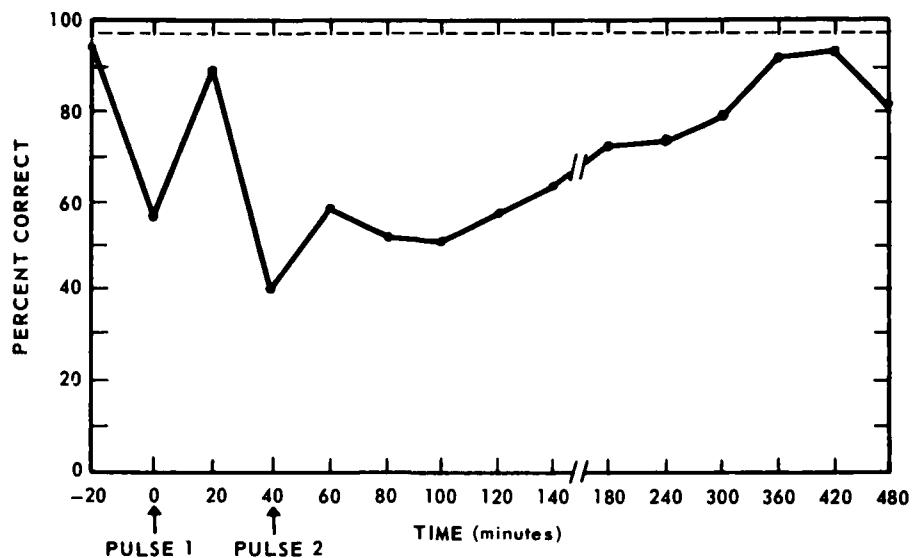


Figure 16. Postirradiation performance after two pulsed exposures separated by 40 minutes (2500 rads).

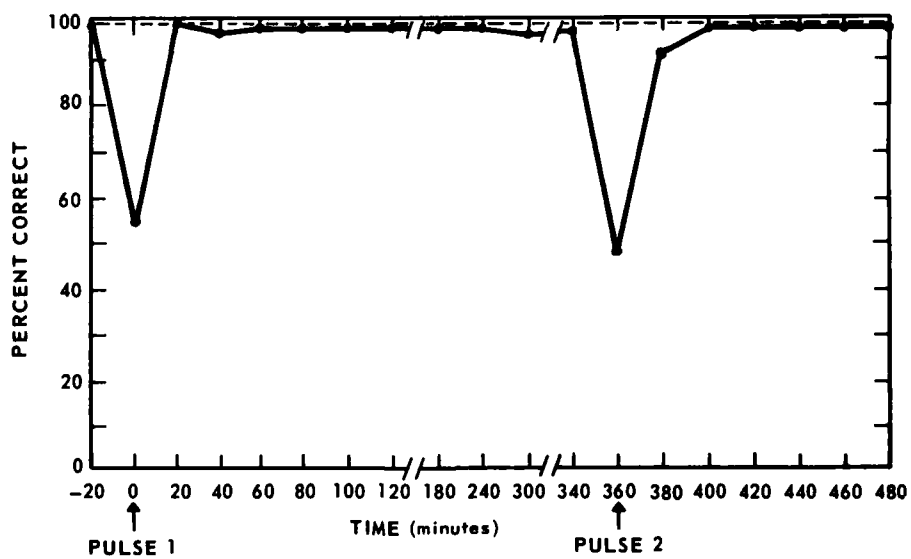


Figure 17. Postirradiation performance after two pulsed exposures separated by 6 hours (1700 + 3500 rads).

An examination of these fractionated dose studies indicates that the performance of the monkeys after the 1700-rad pulse was similar to that observed following a 2500-rad exposure but the 1700-rad dose apparently did not have the protective effect of the first 2500-rad dose when a second pulse is delivered at an interval of 6 hours. The difference in the magnitude of the second pulse (2500 rads versus 3500 rads) may have an important bearing on these data. These results suggest that in monkeys the activation of the mechanism(s) responsible for protection against the second pulse of radiation is a function of both time between exposures and the size of the first pulse relative to the second.

REFERENCES

1. Germas, J. E. and Shelton, Q. H. Performance of the monkey following multiple, supralethal pulses of radiation. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR69-21, 1969.
2. Young, R. W. and McFarland, W. L. The effects of 15,000 rads pulsed gamma-neutron radiation on the behavioral performance of monkeys (*Macaca mulatta*). Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR70-7, 1970.

IDENTIFICATION OF PROMINENT SITES OF RADIATION INJURY AND THEIR RELATIONSHIP TO BEHAVIOR

Principal Investigators: C. L. Turbyfill and R. M. Roudon

Technical Assistance: V. A. Kieffer and B. A. Dennison

The objective of this research is to evaluate the physiological and biochemical changes as they relate to behavior following supralethal doses of radiation.

The cardiovascular response of monkeys² and beagles³ was previously investigated following supralethal doses of mixed gamma-neutron radiation. In the present study physiological and behavioral data following irradiation have been compared to give a better insight into the mechanisms involved in radiation injury.

Trained monkeys surgically implanted to monitor aortic and venous pressures, carotid flow, heart rate and respiratory rate were irradiated with a 2500-rad midline tissue dose of mixed gamma-neutron radiation. The effects of physiological changes

which occurred following irradiation were compared with the animal's ability to perform a learned task and the combined results are presented in Figure 18. An early

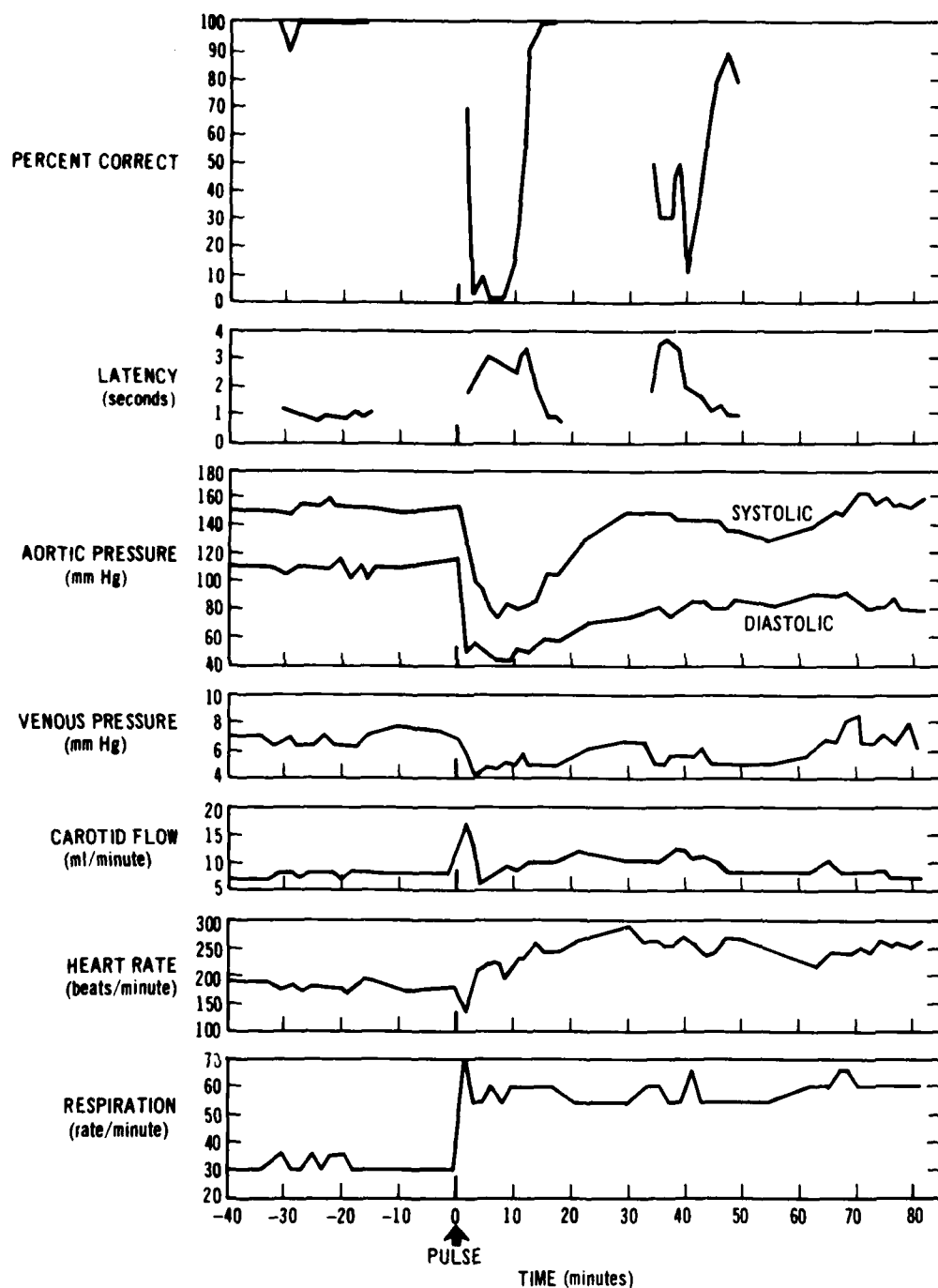


Figure 18. Means for behavior and physiology of six animals receiving a 2500-rad dose of mixed gamma-neutron radiation.

performance decrement (less than 90 percent correct responses) and an acute decrease in aortic pressure were observed within a few minutes following irradiation. The first physiological indication of the ensuing incapacitation period was a rapid fall of diastolic pressure and a decrease in systolic pressure. A second period of decrement was observed at approximately 40 - 45 minutes postirradiation. The respiratory rate was in general significantly increased following irradiation. No significant changes were observed in carotid flow or venous pressure. Changes in the cardiovascular system such as vasodilation of the systemic vasculature and shunting of blood from the cortical areas of the brain may be the causative factors inducing the performance decrement and physiological changes observed in the monkey following irradiation.

A telemetry system was developed that is capable of recording the electrocardiogram of unrestrained animals in an intense radiation environment.¹

REFERENCES

1. Kieffer, V. A. and Turbyfill, C. L. Electrocardiography in a radiation environment by the use of telemetry. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Technical Note TN70-6, 1970.
2. Turbyfill, C. L., Kieffer, V. A. and Dewes, W. A. Cardiovascular response of monkeys to supralethal doses of mixed gamma-neutron radiation. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR70-10, 1970.
3. Turbyfill, C. L., Thorp, J. W. and Wise, D. Cardiovascular response of beagles to a supralethal dose of mixed gamma-neutron radiation. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR70-4, 1970.

HIPPOCAMPAL ELECTRICAL ACTIVITY AND VOLUNTARY MOTOR MOVEMENT IN THE RAT

Principal Investigator: *W. L. McFarland*

Collaborator: *H. Teitelbaum, University of Maryland*

Preliminary studies were conducted to test the hypothesis that a specific part of the brain, the hippocampus, is involved in voluntary motor activity in the rat.¹

Hippocampal and cortical bioelectrical activity and bodily movements were monitored in water-deprived naive rats who were subsequently trained to increase gross voluntary muscular activity beyond base-line levels by running back and forth in a testing chamber for water reward. After the conditioned hyperactivity had reached a stable level, extinction procedures were started by removing the water reward. Hippocampal and cortical signals were recorded on both a paper chart and magnetic tape with the hippocampal signals being directed as well through a band-pass filter set to pass only 6-Hz frequencies.

Changes in behavior from base line to conditioning to extinction are shown in histogram form on the left of Figure 19. Fifty 5-second epochs were picked at random from each phase and epochs categorized according to the number of gross activity counts contained in them. For example, 46 of the 50 epochs in the base-line period contained four or fewer counts and only one epoch contained between 25 and 29 counts. Note the large increase in mean activity level during conditioning from a base-line mean of 1.4 counts per 5-second epoch to a mean of 23.6 counts per epoch. Furthermore, as a consequence of extinction, the behavior of the animal approaches the relative inactivity recorded from the naive animal. As the activity level changed from that at base line to conditioning to extinction, there were corresponding changes in the bioelectric activity recorded from the hippocampus in the different phases of this experiment. This change in hippocampal electrical activity is computed by means of a power spectral density program and shown on the right side of Figure 19 by the representative spectral density plots corresponding to each phase of training. In these plots the area under the curve represents 100 percent of the total power for a 5-second epoch of hippocampal EEG data. During the base-line period there tend to be several peaks in the spectrum, with the largest ones in the region of 2-10 Hz. During conditioning there is a marked shift in distribution of intensity with a large peak appearing at 7 Hz. In the extinction phase a peak remains in the 4- to 7-Hz range, but other peaks seen in the base-line record are beginning to reappear.

Analysis of these recordings revealed that changes in electrical activity of the hippocampus occurred in phase with changes in bodily activity. There were no obvious changes in cortical electrical activity.

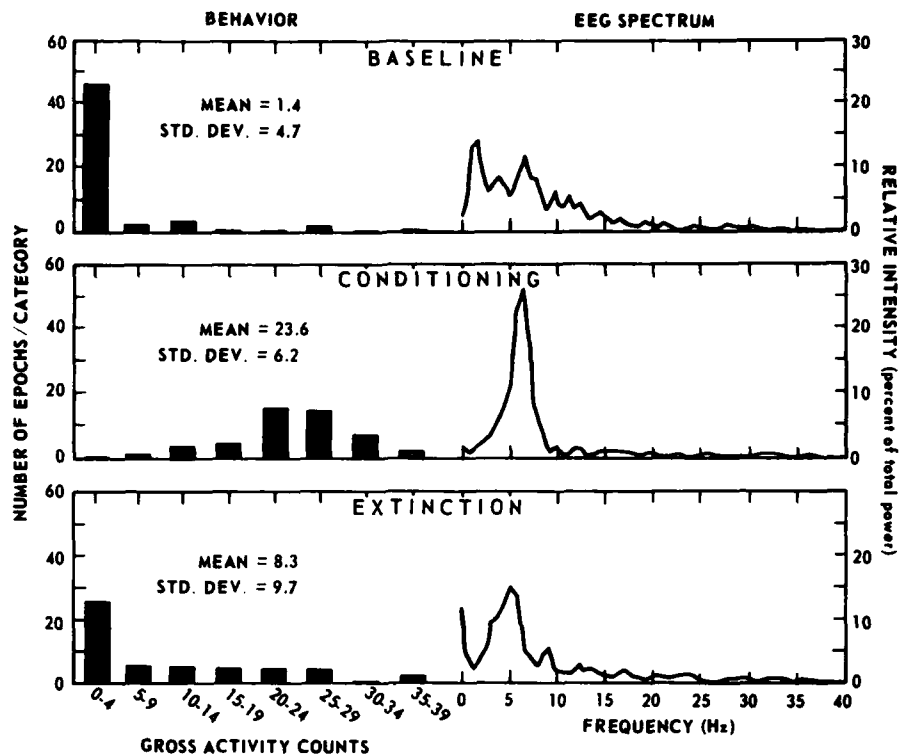


Figure 19. Summary of behavioral changes and hippocampal spectral characteristics during the three phases of training. Explanation of derivation of measures is in the text.

REFERENCE

1. McFarland, W. L. and Teitelbaum, H. Hippocampal electrical activity and voluntary motor movement in the rat. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Technical Note TN70-3, 1970.

RECOVERY AND RESIDUAL INJURY OF THE HEMATOPOIETIC SYSTEM IN IRRADIATED MAMMALS

Principal Investigators: S. J. Baum and D. E. Wyant

Technical Assistance: J. L. Atkinson and M. Neal

In a previous report¹ an oscillatory postirradiation erythropoietic recovery pattern was demonstrated in dogs. These oscillations could have their origin from the interplay of inhibitory and stimulatory factors responsible for the maintenance of the primitive stem cell population or they could be due to an intermittent production in erythropoietin which would affect the release of erythropoietin responsive cells. The objective of this study was to investigate the origin of the postirradiation recovery pattern.

The polycythemic dog was selected for this research effort since its erythropoiesis is stimulated by the administration of a known concentration of exogenous erythropoietin. This eliminates the possibility of a periodic production of endogenous erythropoietin. Dogs made polycythemic by the infusion of donor erythrocytes were exposed to 150 rads of mixed gamma-neutron radiation. The ⁵⁹Fe incorporation into newly formed erythrocytes in response to the administration of 300 units of erythropoietin was measured for 3 weeks thereafter. Previous tests in this Institute have shown that the iron incorporation in polycythemic unirradiated dogs is reduced from a normal 71 percent to 26 percent. The administration of 300 units of erythropoietin returns it to normal.

The hematocrits depicted in Figure 20 clearly indicate that all dogs were polycythemic at the time of erythropoietin and radioiron administration. Figure 21 shows that after irradiation iron incorporation in response to erythropoietin is reduced to 17 percent of control values. Thereafter, several oscillations are noted throughout the 3-week testing period until near recovery is achieved. The highest value of 87 percent was observed on the 15th postirradiation day. Since this oscillatory pattern of erythropoietic recovery in the dogs was obtained in response to the stimulation of an administered constant concentration of erythropoietin it is postulated that it originates in the production of cells in a more primitive population not yet responsive to the hormone.

REFERENCE

1. Baum, S. J. and Wyant, D. E. Hematopoietic recovery in irradiated dogs. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR70-2, 1970.

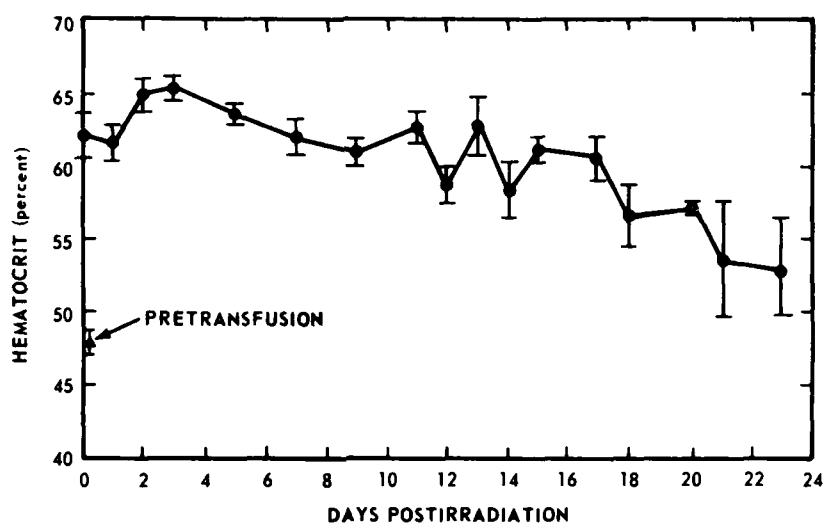


Figure 20. Hematocrits and standard error for polycythemic dogs exposed to 150 rads mixed gamma-neutron radiation.

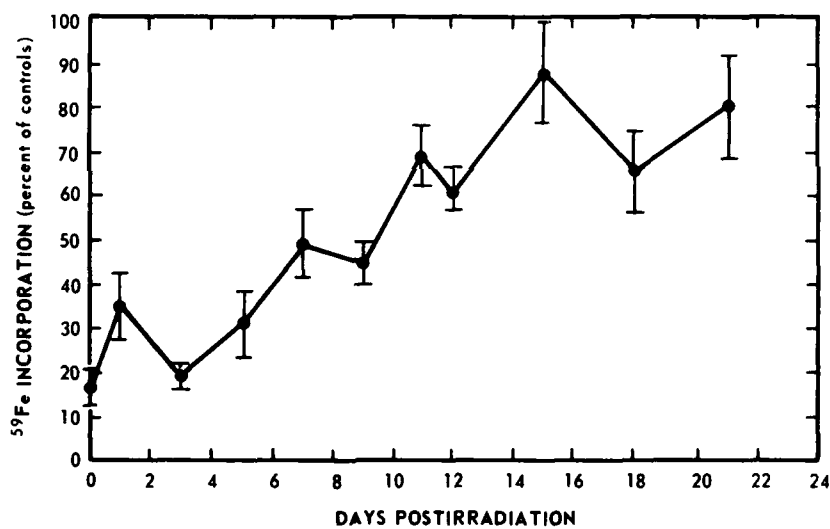


Figure 21. Erythrocytic stem cell recovery in polycythemic dogs exposed to 150 rads mixed gamma-neutron radiation.

◆◆◆◆◆◆◆◆◆◆

MOLECULAR STUDIES OF CELLULAR AND SUBCELLULAR DAMAGE IN THE IRRADIATED ANIMAL

Principal Investigator: G. N. Catravas

Technical Assistance: C. G. McHale

The objective of this research is to identify the sites of damage produced in the fatty acid synthesizing enzyme system in the liver of animals exposed to ionizing radiations and the biological factors which influence it.

Results of early experiments on the effects of different qualities of ionizing radiations on the fatty acid synthesizing liver enzyme system indicated that when rats were exposed to x rays or to ^{60}Co gamma rays, the activity of the enzyme system was greatly enhanced. In contrast, when the animals received the same dose of mixed gamma-neutron radiation the activity of the same enzyme system was inhibited. This inhibition was more pronounced when the neutron to gamma ratio was increased or when a 14 MeV neutron generator was used as the radiation source.

It was assumed that these differences in activity change could be due to neutrons causing a different biological damage as compared to that of the electromagnetic radiations. To investigate this possibility, a series of experiments were performed in which groups of rats were exposed to different doses of x rays or of mixed neutron-gamma (neutron to gamma ratio of 7 to 1) radiation and sacrificed 3 or 24 hours post-irradiation. The livers were excised and cell-free homogenates containing the fatty acid synthesizing enzyme system were prepared. The synthesized fatty acids were chemically isolated and their radioactivity was determined in a liquid scintillation counter. The amount of radioactivity found in the isolated fatty acids, which is a measure of the activity of the enzyme system under study, was calculated. The results are shown in Figure 22 where it can be seen that in x irradiated rats sacrificed 3 hours after exposure, the activity of the fatty acid synthesizing liver enzyme system increases with radiation dose, reaches maximal values at doses between 1200 and 2000 rads and then declines. In animals sacrificed 24 hours postirradiation, the effect is much more pronounced with a more than thirteenfold enhancement of enzymic activity occurring at radiation doses between 1200 and 2000 rads. A similar response of enzymic activity change was observed in rats exposed to mixed neutron-gamma radiation and sacrificed 3 hours after exposure; however, maximal values were observed at much lower radiation doses. Higher doses resulted in impairment of the activity. In animals sacrificed 24 hours after exposure, the pattern of activity change is similar although the stimulation is much smaller.

To determine to what extent the activities of individual enzymes involved in fatty acid biosynthesis are affected by irradiation of the animal, the enzymes acetyl CoA carboxylase and fatty acid synthetase were investigated. Particle-free liver preparations were obtained from experimental and sham irradiated control rats by centrifugation of the corresponding liver homogenates at 100,000 x g. The clear

supernatants were then assayed for activities using acetyl-1- ^{14}C -CoA and malonyl-1, 3- ^{14}C -CoA as substrates for acetyl CoA carboxylase and fatty acid synthetase, respectively. The activity of acetyl CoA carboxylase was only slightly affected when the animals were exposed to x rays at doses up to 4000 rads and sacrificed 3 or 24 hours after irradiation (Figure 23). However, the activity of this enzyme showed a pronounced inhibition in the livers of animals exposed to mixed neutron-gamma radiation. The activity of fatty acid synthetase was stimulated when the rats were exposed to 800 rads of mixed neutron-gamma radiation and sacrificed 3 or 24 hours after exposure. The effect of x rays on the activity of this enzyme was more pronounced (Figure 24). In rats sacrificed 3 hours after exposure a more than twofold enhancement of activity was observed at doses between 800 and 2000 rads. In animals sacrificed 24 hours post-irradiation the enhancement of activity is much more pronounced. At 800 rads a more than sevenfold stimulation was observed. Following a decline, a further increase of activity was observed in animals exposed to 4000 rads of x rays.

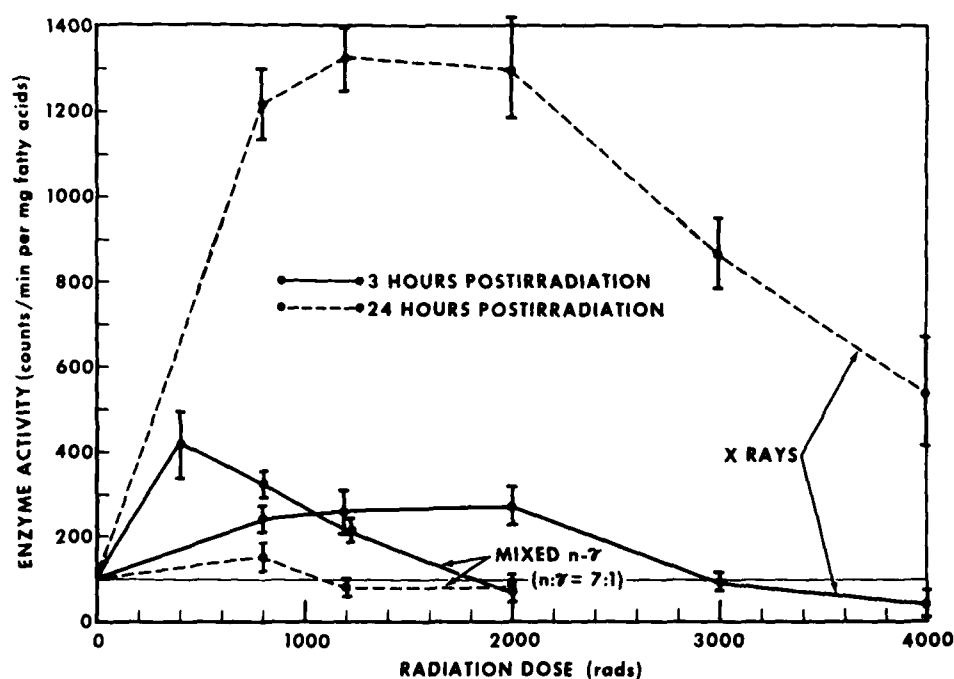


Figure 22. Effect of ionizing radiation on activity of fatty acid synthesizing liver enzyme system.

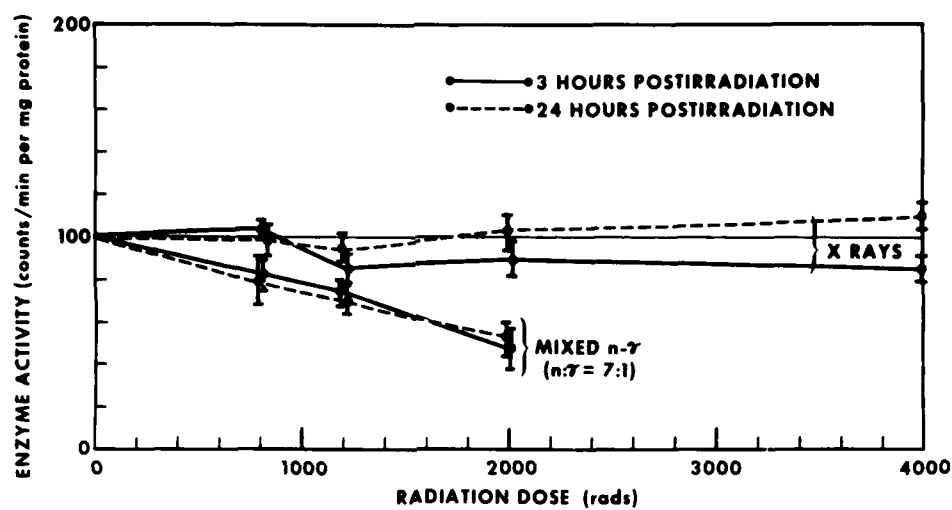


Figure 23. Effect of ionizing radiation on activity of acetyl CoA carboxylase.

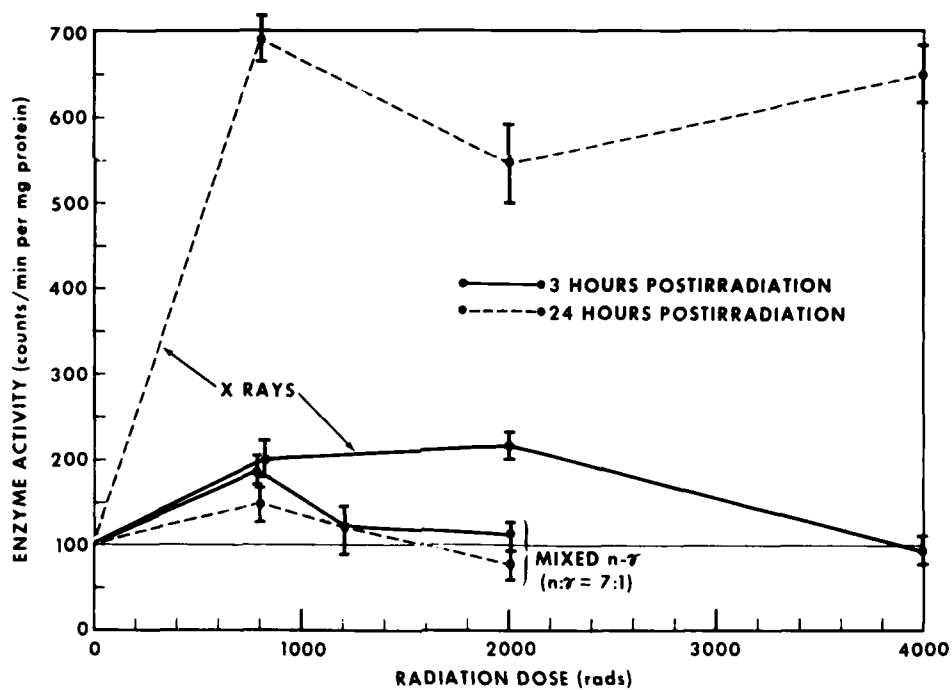


Figure 24. Effect of ionizing radiation on activity of fatty acid synthetase.

These results indicate that the activities of both enzymes are affected by irradiation of the animals and that fatty acid synthetase is much more sensitive to radiation than acetyl CoA carboxylase.

EFFECT OF IONIZING RADIATION ON LIPID PEROXIDATION IN MAMMALIAN CELL MEMBRANES

Principal Investigators: *W. D. Skidmore and G. N. Catravas*

Technical Assistance: *C. G. McHale, E. E. Ricks and O. Z. Williams*

The objectives of this research were to determine factors contributing to the effects of whole-body x irradiation on (1) iron-induced mitochondrial swelling associated with lipid oxidation as an index of injury to the structural integrity of the outer membranes, and (2) oxidative phosphorylation as an index of the functional integrity of the inner membranes in liver mitochondria from rats fed special diets prior to exposure.

Rats were exposed to a dose of 1000 R x rays and sacrificed 24 hours later. Four diets were used in the study: N-T (tocopherol deficient); N (normal fat-supplemented); N-F (fat-free); and normal rat chow.

In comparing the effect of irradiation to that of starvation, it was observed that the lag time for swelling, calculated as t_{50} (time required for a 50 percent decrease in absorbancy when the light scattering was measured at 520 nm) values, decreased as a function of postprandial time. At 24 hours postirradiation, t_{50} values were below those of the unirradiated controls at 24, 48 and 72 hours postprandial times (Figure 25).

In rats on a fat-free diet either fed for 1 hour or starved for 24 hours prior to exposure, the P/O (number of g atoms P incorporated into ATP per g atom oxygen reduced) and RC (respiratory control) values at 24 hours postirradiation are increased above control values for each group. This effect of x irradiation is in the opposite direction to that of starvation which decreased both values significantly below those for the fed group. As a consequence, the P/O and RC values for mitochondria from fed unirradiated rats are similar to those for mitochondria from starved irradiated ones (Table XII). In comparing these results of oxidative phosphorylation to the results of oxidative swelling, the effect of either irradiation or starvation decreased the lag time for swelling.

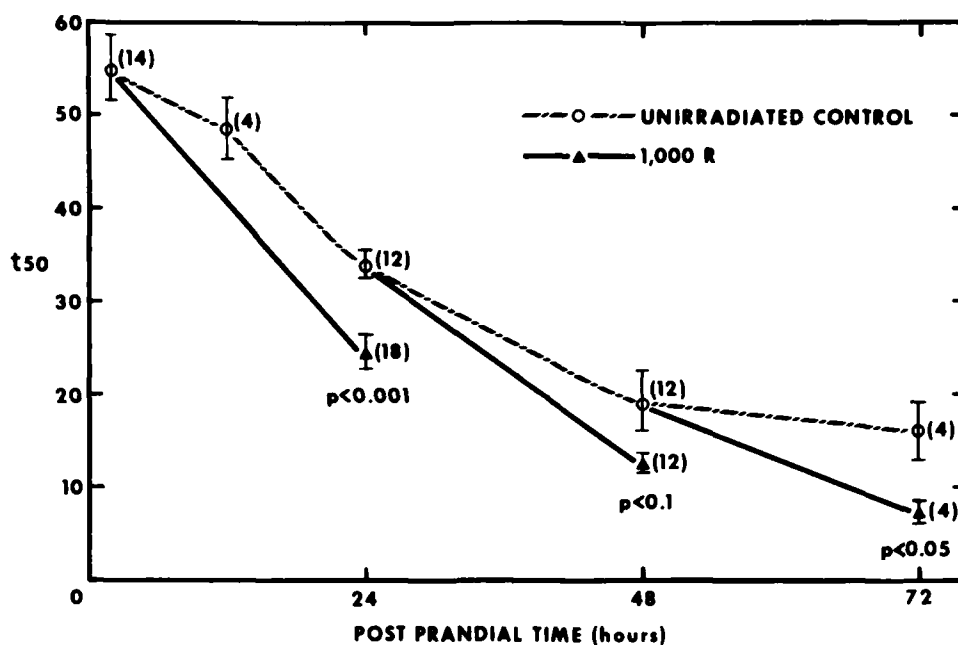


Figure 25. Mitochondrial swelling lag time (♀, fat-free diet, mitochondrial-lysosomal, Fe⁺⁺, 24 hours after x irradiation).

Table XII. Oxidative Phosphorylation [Fat-free Diet, 24 Hours after 1000 R X Rays, Fed or Fasted (24 Hours) before Exposure]

Group	Rats	P/O	RC
A. Fed control	24	1.29 ± 0.03	2.41 ± 0.08
B. 1000 R ("t" test AB)	28	1.44 ± 0.02 <0.001	3.02 ± 0.07 <0.001
C. Starved control ("t" test AC)	19	1.11 ± 0.03 <0.001	1.87 ± 0.08 <0.001
D. 1000 R ("t" test CD)	20	1.29 ± 0.03 <0.001	2.48 ± 0.08 <0.001

In comparing diets, results from the fat-free diet indicated that the lag times for the oxidative swelling were increased above controls, whereas P/O and RC values were lower than controls. In comparison with feeding, effects of starvation were observed as a lower lag time for oxidative swelling and lower oxidative phosphorylation values than for controls. An intercomparison among these effects revealed that oxidative phosphorylation values for mitochondria from fed, unirradiated rats were

similar to those from starved, irradiated ones. The presence of lysosomes in the mitochondrial fractions did not seem to affect the results. However, the lysosomal to mitochondrial ratios of acid phosphatase activities were higher for x irradiated rats than for controls.

EFFECTS OF IONIZING RADIATIONS ON BIOSYNTHESIS OF COMPLEX PROTEINS

Principal Investigator: A. S. Evans

Technical Assistance: F. A. Quinn, K. M. Hartley and M. H. Gobbett

Investigations^{1,2} from this laboratory have indicated that the plasma concentration of protein-bound carbohydrates as neutral hexoses is of significant prognostic value in following the course of radiation injury in mice and dogs. Thus, in both species, the animals which died showed a marked increase in plasma concentration of these bound carbohydrates, while the survivors of identical doses deviated only slightly from their preirradiation base-line values.

A more detailed quantification has now been completed from which numerical ranges of protein concentrations are proposed to indicate good, guarded and poor prognoses for dogs.

Three groups of eight dogs each were given doses of 225, 230 and 400 rads of mixed gamma-neutron radiation, respectively. Blood samples were taken from each dog prior to irradiation and at intervals thereafter for 15 or 20 days or until death intervened. Total protein, protein-bound neutral hexoses, hexosamines, sialic acid, and fucose determinations were made in duplicate on each sample. To eliminate any influence of hemodilution or hemoconcentration, the concentration of each class of carbohydrate was converted to milligrams of carbohydrate per 100 mg biuret protein.

All of the dogs which received 400 rads of mixed gamma-neutron radiation died on or before the 11th day postirradiation. At this overwhelming dose, the protein concentrations of neutral hexoses, sialic acid, and hexosamines as a function of time were similar (Figure 26). Thus, in the seven animals which survived to the 10th or 11th day, these values started to increase on the 6th postirradiation day, continued upward at almost identical rates, and remained high until the death of the animal. These three classes of carbohydrates also followed a similar pattern in the one animal which died on the 4th day postirradiation, but significant increases over preirradiation values did not occur until the day of death.

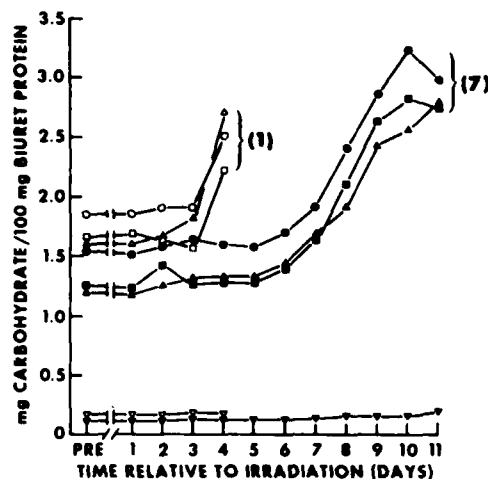


Figure 26. Milligrams protein-bound carbohydrates per 100 mg protein (biuret) in the serum of dogs at indicated times relative to receiving a 400-rad dose of mixed gamma-neutron radiation. Open symbols: died on 4th day postirradiation. Closed symbols: died on day 10 or 11. O, ● = neutral hexoses; Δ, ▲ = hexosamines; □, ■ = sialic acid; ▽, ▼ = fucose. The number of animals represented by each curve is in parentheses.

Seven of the eight dogs which received 230 rads died. In the animals which died, the protein concentrations of neutral hexoses, sialic acid, and hexosamines as a function of time were similar to those seen in the 400-rad experiment (Figure 27A-C). The time courses of these protein-bound carbohydrate concentrations, however, did not parallel one another as closely as in the higher dose group (Figure 26). The one surviving animal of the 230-rad group (Figure 27D) was acutely ill during the 2nd week postirradiation. When the neutral hexoses went above 1.60 mg carbohydrate per 100 mg protein and continued to creep upward, the animal was given a high protein, soft diet, and special hygienic measures were taken to control routes of sepsis in its environment. While it remained high, the protein concentration of neutral hexoses stabilized after the 11th day, and clinical improvement continued for the remainder of the 40-day observation period. At the end of this time, the concentration of protein-bound neutral hexoses had returned to preirradiation levels.

At the lowest dose, 225 rads, three of the eight dogs died during the 40-day observation period. In the animals which died, moderate to marked fluctuations were seen in the protein concentrations of neutral hexoses, sialic acid and hexosamines during the course of the radiation sickness and terminated in a sharp rise shortly preceding death (Figure 28A-C). The five survivors, taken as a group, exhibited no remarkable changes in any of the protein-bound carbohydrate concentrations (Figure 28D). Clinically, however, there was considerable variation in the severity of the radiation sickness among these survivors, and these differences were reflected in the time course of protein-bound neutral hexose concentrations in the individual dogs (Figure 29). In Figure 29, the daily percent change in the protein concentration of each class of carbohydrate from its overall mean for the 15 days was plotted. Thus, while variations follow the same course as a function of time as the raw data, the shift of coordinates permitted direct comparability in that all the carbohydrate concentrations are referenced to the same horizontal line, $y = 0$.

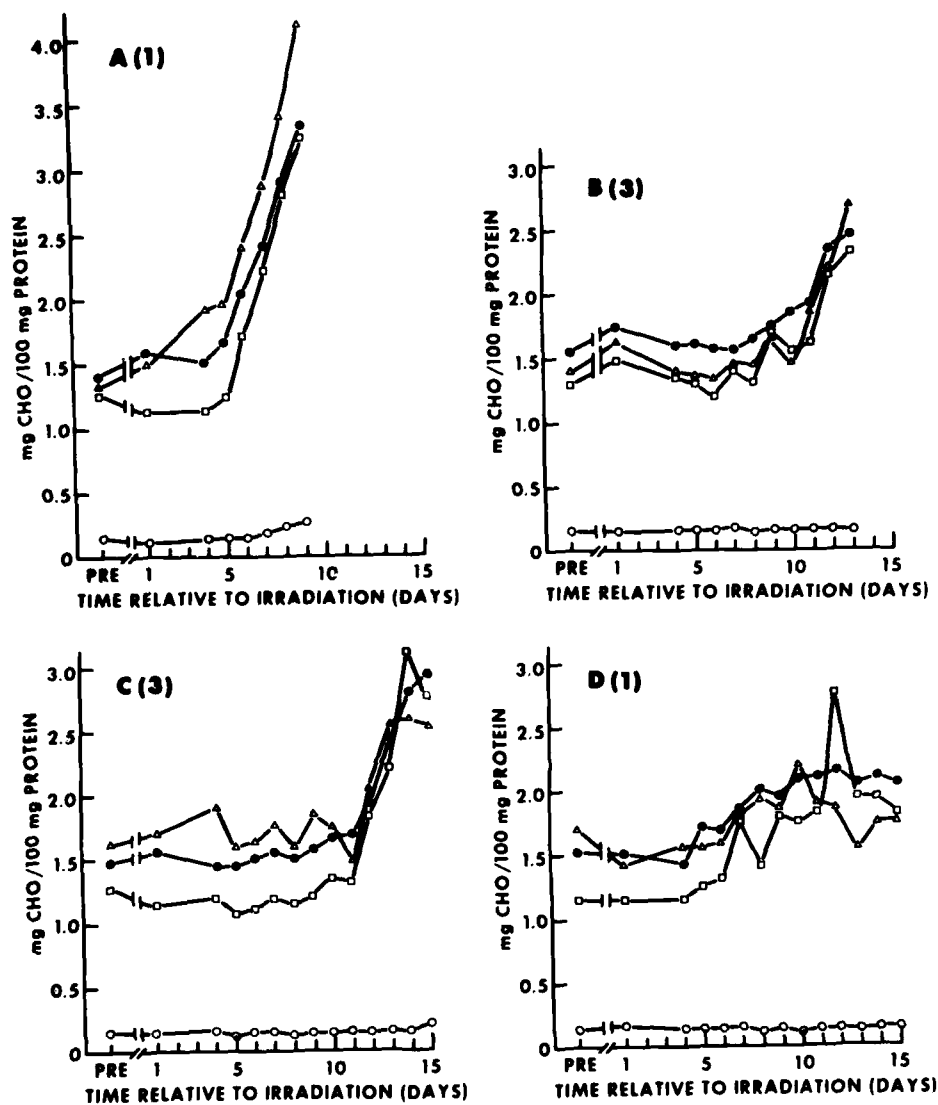


Figure 27. Milligrams protein-bound carbohydrates per 100 mg protein (biuret) in the serum of dogs at indicated times relative to receiving a 230-rad dose of mixed gamma-neutron radiation. A, died on 9th day postirradiation; B, died on day 12 or 13; C, died on day 14 or 15; D, survived (with treatment). ● = neutral hexoses; Δ = hexosamines; □ = sialic acid; ○ = fucose. The number of animals represented by each curve is in parentheses.

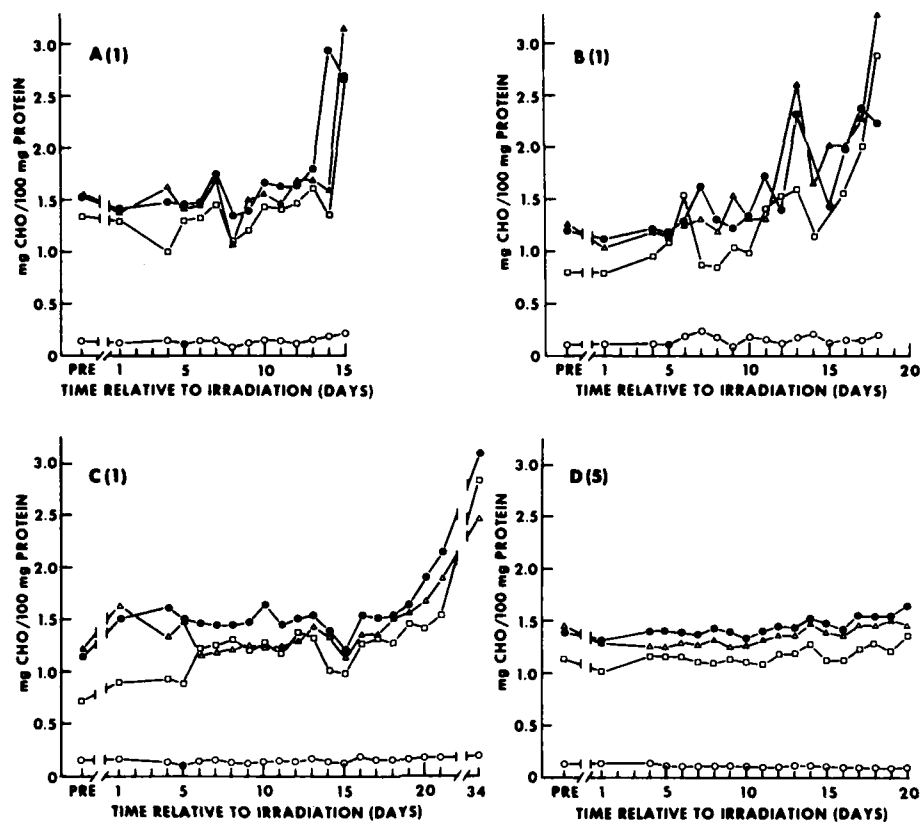


Figure 28. Milligrams protein-bound carbohydrates per 100 mg protein (biuret) in the serum of dogs at indicated times relative to receiving a 225-rad dose of mixed gamma-neutron radiation. A, died on 16th day postirradiation; B, died on day 19; C, died on day 36; D, survived. ● = neutral hexoses; Δ = hexosamines; □ = sialic acid; ○ = fucose. The number of animals represented by each curve is in parentheses.

No remarkable changes were seen in the protein-bound fucose concentration in any of the 24 animals of the three dose groups.

These data, when analyzed in terms of observed clinical condition of the animals and their ultimate fate (survived or died), suggested that the following boundaries could be set.

Protein concentration of neutral hexoses below 1.60 mg per 100 mg protein was evidence of good prognosis. Thus, all the animals which maintained less than 1.60 mg neutral hexoses per 100 mg biuret protein survived without treatment.

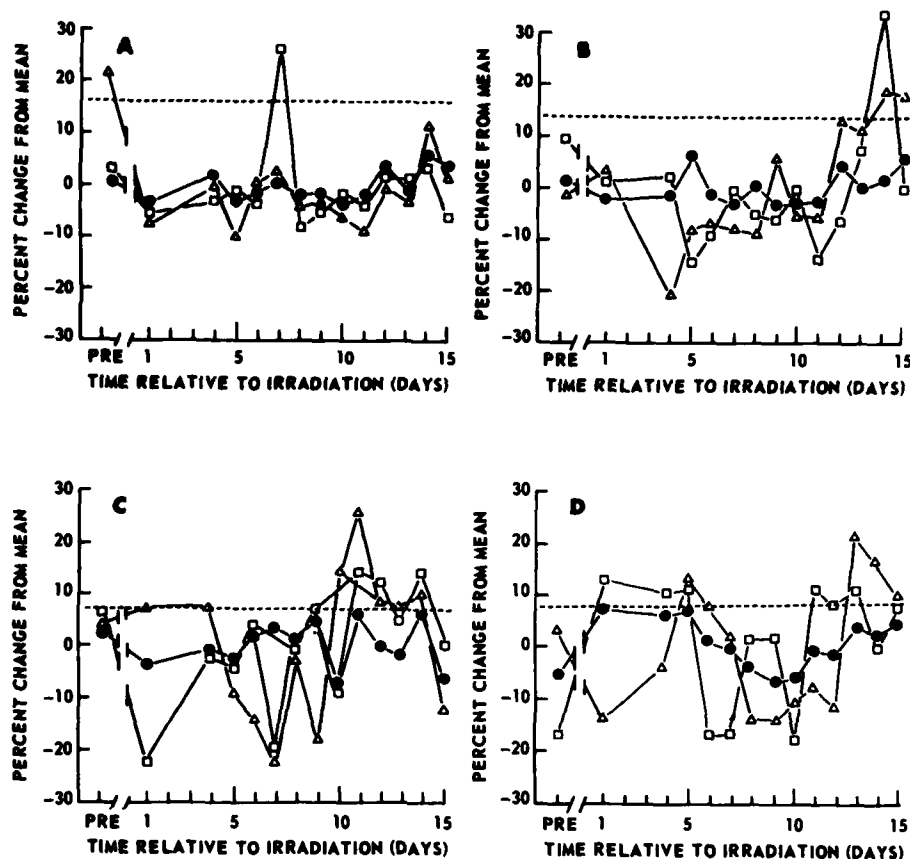


Figure 29. Daily percent change from overall mean of protein concentration of each of various carbohydrates in individual dogs which survived 225 rads of mixed gamma-neutron irradiation with varying degrees of overt clinical symptomatology. The broken line represents the level at which 1.60 mg neutral hexoses per 100 mg protein would fall for each individual. ● = neutral hexoses; Δ = hexosamines; □ = sialic acid.

The interval 1.60 to 1.80 was assigned as the guarded prognosis range. That is, entry into this range within the first 15 days postirradiation presaged impending deterioration, and, if treatment is to be instituted, it should be started immediately. Entry into the guarded prognosis range preceded appearance of overt symptoms.

Protein concentrations of neutral hexoses greater than 1.80 signaled the beginning of the terminal rise and probably indicated that irreversible damage had occurred.

These tentative ranges were tested in nine additional dogs and found to be effective for prediction.

REFERENCES

1. Evans, A. S. Effect of ionizing radiations on distribution of plasma protein-bound neutral hexoses in mice and dogs. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR69-24, 1969. Radiation Res. 43: 152-160, 1970.
2. Evans, A. S., Quinn, F. A., Brown, J. A. and Strike, T. A. Effect of ionizing radiations on total protein-bound neutral hexoses in the plasma of mice. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR68-4, 1968. Radiation Res. 36:128-137, 1968.



EFFECTS OF IONIZING RADIATION ON THE ULTRASTRUCTURE OF MAMMALIAN TISSUES

Principal Investigator: *A. A. René*

Technical Assistance: *J. L. Parker and J. H. Darden*

Ultrastructural and biochemical changes in lysosomes of rat liver following exposure to ionizing radiation were studied. Lysosomes have been identified as distinct intracellular structures bound by a single membrane and containing enzymes. Upon release from the lysosome, these enzymes are capable of causing reparable or irreparable damage. The activity of a number of specific lysosomal enzymes in isolated cells or tissues of animals increases following irradiation.

In an investigation² of the ultrastructural and biochemical changes in lysosomes of rat liver following exposure to ionizing radiation, a marker for acid phosphatase was used to visually correlate the progressive changes in lysosomes with the cellular necrobiotic process postirradiation. The earliest observable change in the lysosomes and/or lysosomal enzymes corresponding with the sequence of fine structural alterations following irradiation suggests that radiation labilizes the lysosomal membrane resulting in a release of enzymes responsible for cell damage. The concentration of the lead phosphate reaction product indicated that the initial action on the lysosome is evidently a "buildup" of hydrolytic enzymes within 2 hours after irradiation followed by a gradual release of the marked enzyme 2 - 24 hours postirradiation as noted by decreased enzyme concentration within the lysosomes (Figure 30). The release of the enzyme appeared to be directly related to an increasing cellular necrobiosis following irradiation.

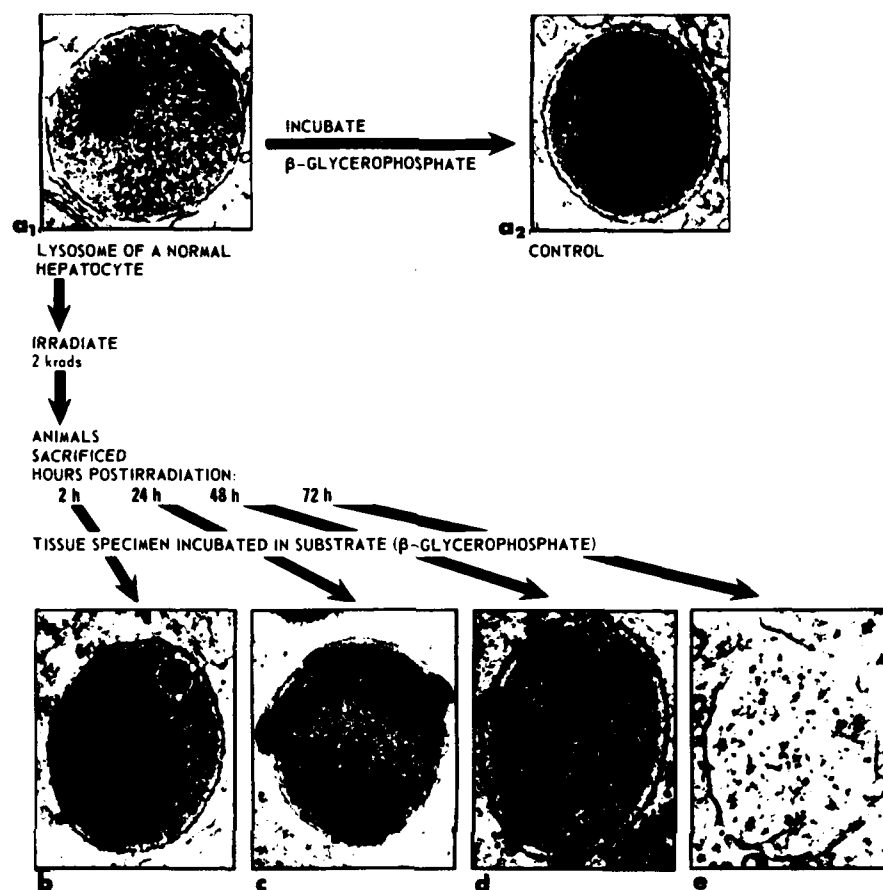


Figure 30. Schematic representation of lysosomal changes following x irradiation. a_1 is a lysosome of a normal hepatocyte. a_2 is a lysosome of a normal hepatocyte after incubation in the substrate medium β -glycerophosphate. b , c , d , and e represent lysosomes at various periods after irradiation, 2, 24, 48 and 72 hours, respectively. At b there is a "buildup" of reaction product which decreases at c and d . There is a loss of membrane competence 72 hours after irradiation (e) allowing the free movement of material across the membrane, resulting in a lysosomal clearing phenomenon.

A further biochemical analysis for acid phosphatase (AcP) was made of soluble protein and lysosomes in liver cells of rats exposed to 2 krad of x rays.¹ The results clearly showed an increase in the acid phosphatase activity in the lysosomes and in soluble protein 2 hours after exposure (Table XIII). This was followed by a decrease in enzyme activity in the lysosomes and a consistent high level in the soluble protein. The results correlated well with the cytochemical studies and showed that, in addition to the radiation-induced increase in number and size of lysosomes, there is a distinct increase of acid phosphatase in the lysosomes themselves. The persistent high level of acid phosphatase in the soluble protein (cytoplasm) was a possible indication of early radiation impairment of the lysosomal membrane.

Table XIII. Enzyme Activity (AcP) in Lysosomal and Soluble Protein Fractions of Liver Homogenates of Control and Irradiated Rats (2 krad) Sacrificed 2 and 72 Hours after Exposure. The Numerical Values Represent Enzyme Activity per Unit of Protein ($\mu\text{g}/\text{ml}$).

Time animals were sacrificed	Lysosome fraction	Percent increase	P values*	Soluble protein fraction	Percent increase	P values*
2 hours postirradiation						
Control	0.80			2.04		
Irradiated	1.81	126	<.01	4.13	102	<.01
72 hours postirradiation						
Control	1.33			2.25		
Irradiated	1.34	<1	>0.5	3.22	43	<.01

* t test

REFERENCES

1. René, A. A., Darden, J. H. and Parker, J. L. Radiation-induced ultrastructural changes in lysosomes. II. Biochemical analysis. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR70-8, 1970.
2. René, A. A., Parker, J. L. and Darden, J. H. Radiation-induced ultrastructural changes in lysosomes. I. Cytochemical analysis. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR69-26, 1969.

♦♦♦♦♦♦♦♦♦♦

EFFECTS OF IONIZING RADIATION ON IMMUNE RESPONSES

Principal Investigators: E. A. Eikman and R. T. Bowser

Technical Assistance: R. T. Brandenburg

This study is designed to enhance the effectiveness of bone marrow transplants by reducing the graft versus host reaction (GVHR) and increasing the hematopoietic precursors. The present approach to the GVHR problem was based upon the fact that the thymus-dependent population of lymphocytes is the component of bone marrow

probably responsible for the GVHR. Experiments were conducted to test the hypothesis that by selectively mobilizing this offending population out of donor organs, the hematopoietic potential remaining in the organ would be increased and the GVHR potential reduced. Transplantation of the mouse spleen was chosen as an appropriate experimental model.

Adult donor mice of the strain C57BL/6J were injected intravenously with Bordetella pertussis vaccine to produce the mobilization of thymus-dependent lymphocytes. By the third day peripheral blood counts showed an eightfold increase in peripheral lymphocytes over control values from saline injected mice. Donor spleens were removed and pooled cell suspensions were prepared and assayed for GVHR and hematopoietic potential separately. The cells were injected into newborn hybrid offspring of the cross between the donor C57BL/6J strain and the DBA/2J strain. This hybrid is designated as the B6D2F₁ strain. An assay of the GVHR which ensues was quantitated by comparing the spleen weight of injected animals with uninjected controls at 9 days. A spleen index was calculated.

The results of values for approximately 150 assays are summarized in Figure 31. This figure shows that the cells from pertussis treated animals cause consistently lower spleen indices; the difference is significant with $p < .01$. Since the dose response curves are parallel it is possible to estimate the GVHR reduction at about eightfold for the dose range tested. This estimate is made by comparing the number of cells required to produce a given degree of GVHR.

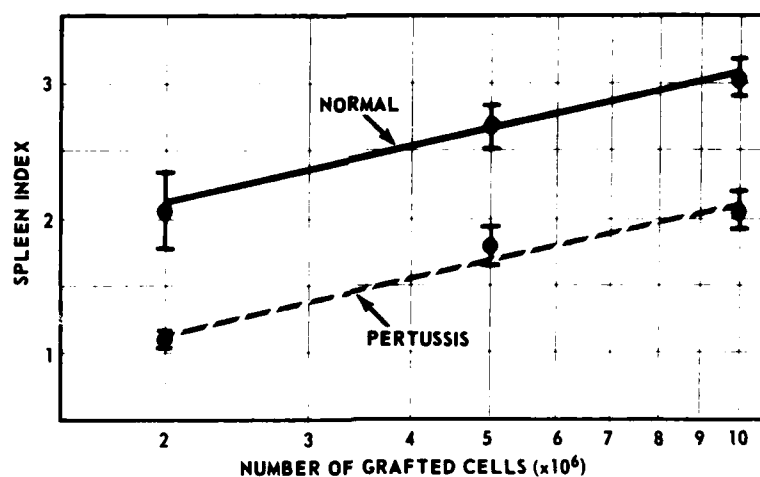


Figure 31. Reactivity of spleen cells from C57BL/6J mice injected into neonatal B6D2F₁ mice.

The hematopoietic capacity of the cell preparation was assayed by injecting cells into lethally irradiated isogenic recipients. Survivors at 10 days were injected subcutaneously with ^{59}Fe . Splenic iron uptake was measured and the nodules representing hematopoietic cell colony proliferation on the surface of the spleen were counted. These nodules were designated as colony forming units (CFU).

Table XIV summarizes the values obtained for radioiron uptake. Significant enhancement of splenic radioiron uptake was noted when cells from pertussis treated donors are compared with the same number of cells from control donors. The results of the CFU assay of hematopoietic potential are summarized in Table XV. Hematopoietic CFU were recognized as nodules greater than .5 mm in diameter on recipient spleens. Again, significant enhancement of hematopoietic activity was noted at both doses examined in the groups receiving cells from pertussis treated donors.

Table XIV. Spleen ^{59}Fe Uptake Counts/Minute $\times 10^{-3}$

Cell number	Donor pretreatment		p
	Pertussis	Saline	
2×10^5	6.0	1.4	<0.01
10^6	33.0	12.0	<0.01
Untreated irradiated control:			0.97
Untreated unirradiated control:			6.3

Table XV. Colony Forming Units per 10^6 Cells Injected

Cell number	Donor pretreatment		p
	Pertussis	Saline	
2×10^5	1.8	0.07	<0.01
10^6	1.7	1.0	<0.05
Untreated irradiated control:			0
Untreated unirradiated control:			0

In general, these data lead to the preliminary conclusion that the pretreatment of donor mice by a single dose of pertussis simultaneously decreases the GVHR and increases the hematopoietic precursors in donor spleens.

◆◆◆◆◆◆◆◆◆◆

ALTERATION OF THE CIRCULATORY AUTOREGULATION OF THE SMALL INTESTINE DURING THE DEVELOPMENT OF THE GASTROINTESTINAL RADIATION SYNDROME

Principal Investigator: J. Kabal

Technical Assistance: L. J. Parkhurst and F. L. Gibson

The objective of this investigation was to measure the radiation-induced direct or indirect changes in the functional integrity of the terminal vascular bed of the small intestine.

To establish the presence and the functional capacity of the autoregulatory mechanism of the small intestinal circulation during the postirradiation period, an in situ dog intestinal loop preparation was used. Beagles were exposed to 1500 rads (midline tissue dose) of mixed gamma-neutron radiation. Hemodynamic parameters of the intestinal loop (ileum, about 10 - 15 grams) were recorded and samples were collected for anatomical (capillary integrity by Microfil techniques) or histological preparations. The irradiated animals were subjected to the procedures 48 and 72 hours after irradiation and their values were compared to control intact beagles.

The results indicate that in the first part of the postirradiation period when the capillary integrity is virtually intact and the hemodynamic parameters are not yet significantly altered, the functional integrity of the intestinal vasculature is already significantly altered. These physiological alterations were measured in response to the administration of norepinephrine, isoproterenol and to bleeding and reinfusion.

At 48 hours postirradiation, the sudden intestinal hemodynamic changes induced by alpha or beta adrenergic agents were less compensated than in nonirradiated controls. At 72 hours postirradiation this compensation had completely disappeared (Table XVI). This phenomenon is closely related to nerve conductance alterations. The intestinal resistance vessels of the irradiated animals did not exert "autoregulatory escape" as the controls did (Figure 32). The hemodynamic values of the intestinal resistance vessels of the irradiated dogs during gradual blood withdrawal and reinfusion also clearly indicated the lack of autoregulation. The hyperemic tendency of the control vessels during reinfusion was completely missing in the irradiated animals. Furthermore, the fluctuation of the intestinal resistance values at 48 and 72 hours postirradiation demonstrated the effect of neurohumoral imbalance (Figure 33).

The findings of the present study indicate that in the development of the postirradiation gastrointestinal syndrome there is a progressive alteration in the functional capacity of the intestinal resistance vessels. The significance of this work is related to the hypothesis that the terminal cardiovascular collapse observed in dogs succumbing to the injury termed the gastrointestinal radiation syndrome might be due to intestinal ischemic shock.

Table XVI. Percentage Vasoactive Compensation of the Intestinal Resistance Vessels after the Administration of Norepinephrine (5 μ g/kg)

Groups	Time intervals (min)	Change in blood pressure (mm Hg)	Change in blood flow (percent)	Percent blood flow change per 100 mm Hg blood pressure change	Vasoactive compensation (percent)
Control	0 - 1	+ 74.6	+ 26.4	+ 35.39	+ 75.2
	1 - 2	- 47.8	- 4.2	- 8.78	
48 hours postirradiation	0 - 1	+ 80.2	+ 50.0	+ 62.34	+ 42.4
	1 - 2	- 60.2	- 21.6	- 35.88	
72 hours postirradiation	0 - 1	+ 83.0	+ 57.4	+ 69.16	- 0.10
	1 - 2	- 59.3	- 41.3	- 69.65	

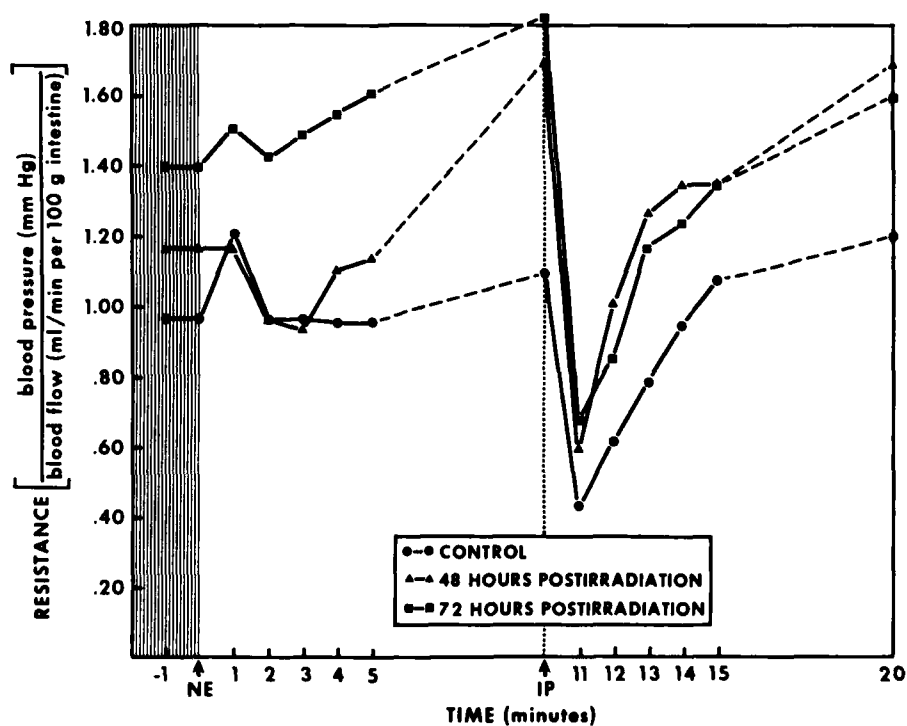


Figure 32. Intestinal vascular resistance pattern in minute intervals after the injection of norepinephrine (NE) and isoproterenol (IP).

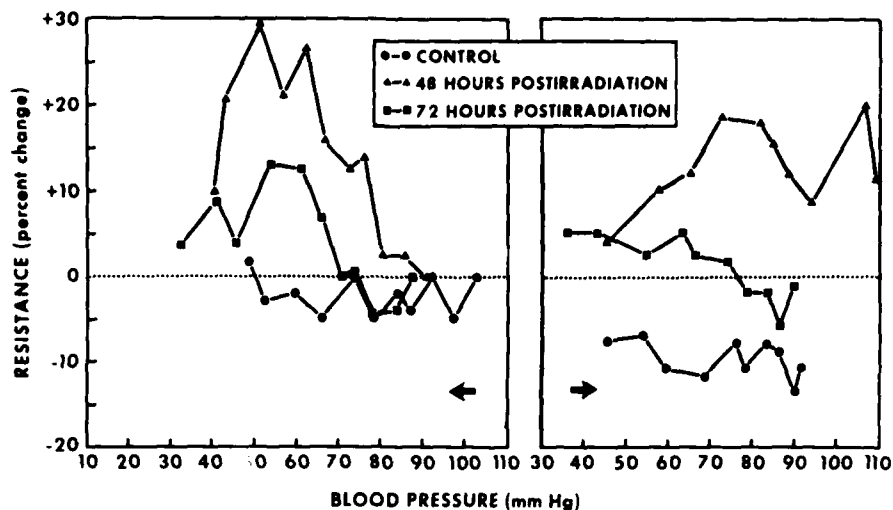


Figure 33. Intestinal vascular resistance changes in percentage of the prehemorrhagic values according to the blood pressure alterations during gradual blood withdrawal ← and reinfusion → periods. Each point represents the average percent change of the intestinal resistance induced by subsequent 20 ml blood volume alterations.

◆◆◆◆◆◆◆◆◆◆

HEPATIC CYSTICERCOSIS IN A MOUSE COLONY

Principal Investigators: *M. W. Balk and S. R. Jones*

Technical Assistance: *G. D. Lee, P. J. Ellis and J. A. Laird*

On 10 February 1970, 225 male albino ICR mice, 6 to 8 weeks old, were received and placed in quarantine for a period of 2 weeks. During this time 7 mice died, all showing signs of diarrhea. Failing to find a pathological agent on bacteriological or parasitological examination, it was decided to sacrifice the entire group because the diarrhea was evident among 18 other mice.¹ A randomly selected group of these mice were then euthanatized and cultures were taken in an attempt to identify the causative agent. At necropsy, parasitic cysts were found in the livers of several animals. With this finding, all of the remaining animals were necropsied and 49 of the 210 animals contained hepatic cysts (an incidence of 23.3 percent). The number of cysts per liver varied from one to seven with the majority containing multiple cysts (Figure 34).



Figure 34. Multiple hepatic cysts of Cysticercus fasciolaris in a mouse. X 5

The diameter of these spherical bladder-like cysts varied from 4 to 9 mm. The dimensions of the single white larva within each cyst varied in length from 3 to 6 mm, and in width from 1.5 to 2.0 mm (Figure 35). The rostellum of these larvae contained 4 suckers and 32-40 hooklets (Figure 36). Measurements of the large and small hooklets were compatible with those given for Cysticercus fasciolaris.²



Figure 35. Incised hepatic cyst showing larva (arrow) and attached membranes of Cysticercus fasciolaris.

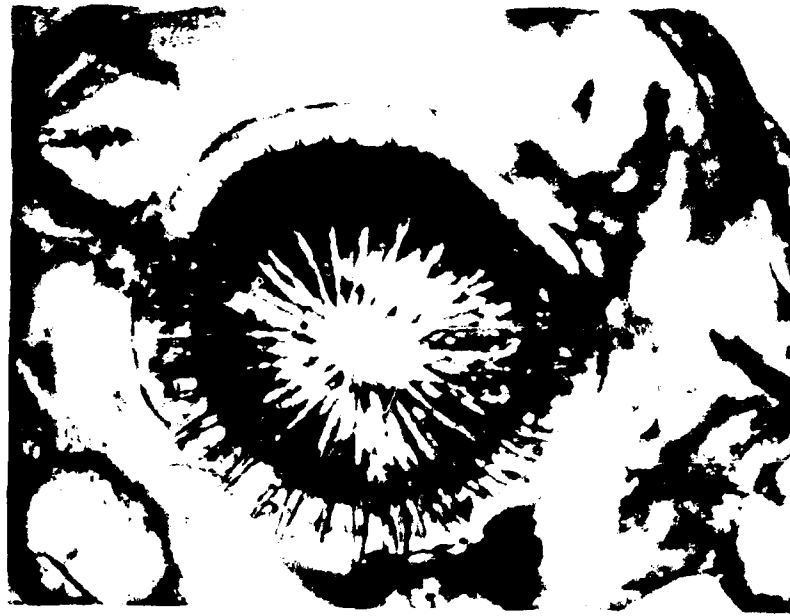


Figure 36. The rostellum of the larva Cysticercus fasciolaris showing four suckers and large and small hooklets. Squash preparation. X 60

Histological lesions consisted of marked hepatic parenchymal displacement by the cysts and an intense zone of granulomatous inflammation surrounding each cyst. Hepatic cord cells adjacent to the granulomatous zone were moderately compressed. Sections through larval scolices within the cysts revealed the presence of hooklets which were anisotropic in polarized light.

After consulting the breeder, we were informed that some neighborhood cats had gained access to the mouse holding facility via the refuse conveyer system and had obviously contaminated the feed and/or bedding with eggs of Taenia taeniaeformis. Since this time, measures have been taken to exclude unwanted animals from the facility.

The Cysticercus fasciolaris (syn. Strobilocercus fasciolaris) is the larval stage of the cestode Taenia taeniaeformis (syn. Hydatigera fasciolaris, Taenia crassicollis). This tapeworm is found in the intestinal tract of the cat, dog, and other carnivores and sheds eggs which are infective in the mouse, rat and occasionally the rabbit, squirrel and muskrat. When the intermediate host ingests the egg, the released oncospheres penetrate the intestinal tract, reach the liver and grow to the infective stage after about 30 days. The larvae appear harmless in the intermediate host even when present in large numbers; however there are reports of hepatic sarcomas and true sarcomatous proliferation of the connective tissue surrounding these parasitic cysts.

The life cycle is completed when the primary host (cat, dog, etc.) ingests the intermediate host and the larvae within the hepatic cysts then de-encyst and infect the carnivore.

Taenia taeniaeformis is a common tapeworm in cats that have access to the intermediate host. Therefore, all rodent breeders should be aware that cats around their facility are potentially able to infect their rodents with the larval stage (Cysticercus fasciolaris) if the feed and/or bedding is accessible to them. For this reason all rodent facilities should not only be cat-proof, but also be escape-proof for the rodents to prevent attracting the roving feline.

REFERENCES

1. Balk, M. W. and Jones, S. R. Hepatic cysticercosis in a mouse colony. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Technical Note TN70-2, 1970.
2. Soulsby, E. J. L. Textbook of Veterinary Clinical Parasitology, Vol. I, Helminths, p. 126. Philadelphia, Pennsylvania, F. A. Davis Co., 1965.



NEUTRON FLUENCE TO KERMA FACTORS AND MASS ENERGY TRANSFER COEFFICIENTS FOR MATERIALS IN REACTOR NEUTRON FIELDS

Principal Investigator: B. E. Leonard

Collaborators: D. M. Verrelli, R. T. Devine and P. A. Berardo

It was necessary to estimate the neutron kerma in tissue from the continuous spectrum of the AFRRI-TRIGA reactor. International Commission on Radiation Units and Measurements (ICRU) Report 13 provides data on the kerma deposited by mono-energetic neutrons in various materials.¹ Calculations have been performed of thermal (Maxwellian), intermediate (1/E) and fast (Watt fission) spectra averaged fluence to kerma factors and mass energy transfer coefficients. The basic data for these calculations were from the Neutron Fluence-to-Kerma Conversion Factor Library³ available from the U. S. Atomic Energy Commission, Radiation Shielding Information Center (RSIC). The computer code AVKER,⁴ which permits 1/E spectra weighting, was modified to permit Maxwellian thermal and Watt and Terrell fission spectra weighting. The spectra weighted kerma per unit neutron fluence (K/φ) and

mean mass energy transfer coefficients ($\overline{\mu_K/\rho}$) were obtained by numerical integration of

$$K/\phi = \frac{\int \frac{d\phi(E)}{dE} E \frac{\mu_K(E)}{\rho} dE}{\int \frac{d\phi(E)}{dE} dE} \quad (1)$$

and

$$\overline{\mu_K/\rho} = \frac{\int \frac{d\phi(E)}{dE} \frac{\mu_K(E)}{\rho} dE}{\int \frac{d\phi(E)}{dE} dE} \quad (2)$$

Values of the resultant quantities for the three neutron spectral regions for 42 materials of interest to dosimetry and radiobiology were reported.² If it is possible to characterize a neutron spectrum by these distributions in the three spectral regions and if an estimate of the magnitudes of the total fluence in each region (ϕ_{TOT}) can be obtained by calculation or measurement, then the kerma (initial kinetic energies of all the charged particles liberated by the neutron field in a differential mass of the material) may be estimated by

$$\begin{aligned} K \approx & (K/\phi)_{THERMAL} \times (\phi_{TOT})_{THERMAL} \\ & + (K/\phi)_{INTERMEDIATE} \times (\phi_{TOT})_{INTERMEDIATE} \\ & + (K/\phi)_{FAST} \times (\phi_{TOT})_{FAST} . \end{aligned} \quad (3)$$

These data and equation (3) were used to compute the estimated neutron kerma in dosimetric materials used at the AFRRI to determine neutron and gamma ray doses at various points in the AFRRI-TRIGA reactor exposure rooms. Values of ϕ_{TOT} were estimated from neutron spectrum measurements by the activation foil method. Table XVII provides the estimated tissue-equivalent kerma from the reactor at 100 cm from the core center line.

Table XVII. Calculated and Measured Neutron Kerma from the AFRRI-TRIGA Facility at 100 cm

Component	Muscle tissue kerma (ergs g ⁻¹ sec ⁻¹ W ⁻¹)	
	Calculated	Measured
Thermal (Maxwellian)	9.613 x 10 ⁻⁶ *	
Intermediate (1/E)	2.265 x 10 ⁻³	
Fast (fission)	1.643 x 10 ⁻²	
Total	1.870 x 10 ⁻²	1.787 x 10 ⁻²

* The thermal spectrum from the reactor is modified using a cadmium and gadolinium shield

The measured value of neutron muscle tissue kerma was obtained using paired 50 cm³ muscle tissue-equivalent and graphite ionization chambers.

The accuracy of the fluence spectrum components is probably to within about 15 percent. The uncertainty in the neutron fluence to kerma conversion factors is mainly from uncertainties in neutron cross sections which are in some instances as much as 10 percent. Thus the overall accuracy for the computed kerma at 100 cm is to within about 18 percent. The difference between the measured and computed tissue kerma given in Table XVII is 7 percent.

REFERENCES

1. International Commission on Radiation Units and Measurements (ICRU) Report 13. Neutron fluence, neutron spectra and kerma. Washington, D. C., 1969.
2. Leonard, B. E. Neutron fluence-to-kerma factors and mass energy transfer coefficients for various materials in reactor neutron fields. Trans. Am. Nucl. Soc. 13(2):885-887, 1970.
3. Ritts, J. J., Solomito, M. and Stevens, P. N. Calculation of neutron fluence-to-kerma factors for the human body. Nucl. Appl. Technol. 7:89-99, 1969.
4. Solomito, M., Ritts, J. J. and Claiborne, H. C. AVKER: A program for determining neutron kerma factors for use in energy deposition calculations. Oak Ridge, Tennessee, Oak Ridge National Laboratory Report TM-2558, 1969.

♦♦♦♦♦♦♦♦♦♦

INDEX TO PRINCIPAL INVESTIGATORS

	Page
Balk, M. W.	53
Baum, S. J.	34
Bowser, R. T.	48
Catravas, G. N.	36, 39
Chaput, R. L.	1, 3
Curran, C. R.	21
Doyle, T. F.	17
Eikman, E. A.	48
Evans, A. S.	41
George, R. E.	1
Jones, S. R.	14, 53
Kabai, J.	51
Kovacic, R. T.	3
Leonard, B. E.	56
McFarland, W. L.	25, 32
Miletich, D. J.	17
René, A. A.	46
Roudon, R. M.	29
Skidmore, W. D.	39
Strike, T. A.	17
Thorp, J. W.	1, 6
Turbyfill, C. L.	29
Turns, J. E.	17
Verrelli, D. M.	1
West, J. E.	10
Wyant, D. E.	34
Young, R. W.	6, 25

UNCLASSIFIED

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) Armed Forces Radiobiology Research Institute Defense Atomic Support Agency Bethesda, Maryland 20014		2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	
		2b. GROUP N/A	
3. REPORT TITLE ANNUAL RESEARCH REPORT 1 July 1969 -- 30 June 1970			
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)			
5. AUTHOR(S) (First name, middle initial, last name)			
6. REPORT DATE		7a. TOTAL NO. OF PAGES 61	7b. NO. OF REFS
8a. CONTRACT OR GRANT NO.		9a. ORIGINATOR'S REPORT NUMBER(S) ARR-4	
b. PROJECT NO.		9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	
c.			
d.			
10. DISTRIBUTION STATEMENT Approved for public release; distribution unlimited			
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY Director Defense Atomic Support Agency Washington, D. C. 20305	
13. ABSTRACT This report contains a summary of the research projects of the Armed Forces Radiobiology Research Institute for the period 1 July 1969 to 30 June 1970.			

DD FORM 1473

1 NOV 65

(PAGE 1)

S/N 0101-807-6801

UNCLASSIFIED

Security Classification

Security Classification

14

KEY WORDS

LINK A

LINK 8

LINK C

ROLE

WT

ROLE

WT

NAME	ROLE
Mr. J. Edgar Hoover	Director
Mr. Clegg	Chief of Bureau
Mr. Glavin	Chief of Bureau
Mr. Ladd	Chief of Bureau
Mr. Nichols	Chief of Bureau
Mr. Rosen	Chief of Bureau
Mr. Tracy	Chief of Bureau
Mr. Carson	Chief of Bureau
Mr. Egan	Chief of Bureau
Mr. Gurnea	Chief of Bureau
Mr. Hendon	Chief of Bureau
Mr. Pennington	Chief of Bureau
Mr. Quinn	Chief of Bureau
Mr. Nease	Chief of Bureau
Mr. Gandy	Chief of Bureau

WT

UNCLASSIFIED

Security Classification

DATE
ILMEI